Erythrocyte Oxidative Stress, Salivary Cortisol, and Oxygen Consumption with Low-Intensity Blood Flow Restricted Resistance Exercise Before and After Training

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Definition of Terms

Reactive Oxidative Species (ROS)- chemically reactive substances containing oxygen with an unpaired electron in their outer layer

Lipid Peroxidation- oxidative degradation of lipids where ROS “steals” electrons from lipids in cell membranes, results in formation of cis turns, and in degradation of cell membranes

Phospholipid- component of cell membranes with a phosphate group head and two fatty acid tails, form lipid bilayer in cell membranes

Protein Carbonyls- protein with modified of amino acid side chain due to oxidative stress

Hemoglobin- iron-containing oxygen-transport protein in red blood cells

Spectrin- protein that forms cellular scaffolding on interior side of cell membrane

Excess Post-Exercise Oxygen Consumption (EPOC)- increased oxygen consumption following exercise

Oxygen Debt- difference between oxygen consumed and metabolic cost of exercise

Minute Ventilation- volume of air inhaled or exhaled by a person per minute

Glucose Transporter Type 4 (GLUT-4)- insulin-regulated glucose transporter in cell membrane that also responds to muscle contraction and stretching by translocating to cell membrane

Buffy Coat- portion of anticoagulated blood sample that contains white blood cells and platelets

Abstract: This study compared oxidative stress in erythrocytes, salivary cortisol, and excessive post-exercise oxygen consumption (EPOC) during traditional high-intensity back squats and low-intensity back squats with blood flow restriction (BFR) as well as the effects of two weeks of training on these markers. Nineteen trained and untrained males participated in the study and 16 (8 trained) completed the two-week training program. When comparing Low-Intensity with BFR to Traditional High-Intensity, plasma lactate values were significantly increased immediately after exercise compared to all other timepoints ($p<.013$), the V(C=O) bond significantly decreased in untrained individuals at the 30 minute and 24 hours post exercise timepoints, and the immediate post-exercise timepoint was significantly increased in Untrained individuals with low-intensity exercise with BFR and the 24 hours post exercise timepoint was significantly increased in Trained individuals with low-intensity exercise with BFR. There were significant increases in salivary cortisol with low-intensity back squats with BFR 30 minutes post-exercise before training ($p<0.05$), but not with high-intensity back squats or with low-intensity back squats with BFR after training. When comparing acute changes before and after two weeks of low-intensity exercise with BFR, plasma total spectral area decreased at 30 minutes post-exercise ($p=0.004$), and plasma lactate immediately after exercise was significantly higher than 30 minutes post exercise ($p=0.022$) and 24 hours post exercise ($p=0.005$). The V(C=H):Vas(CH_3) ratio increased with training in Untrained individuals, but decreased in trained individuals and the V(C=O) bond was significantly reduced at 30 minutes post-exercise compared to 24 hours post exercise. There were no significant differences between conditions or with training in EPOC magnitude or duration, but both of these measures were significantly different between trained and untrained individuals ($p<0.05$). Additionally, two weeks of training significantly increased three-repetition maximum strength in both trained and untrained participants, although untrained participants increased significantly more than trained participants.

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Part 1

Introduction 1: Effects of Acute Traditional and Low-Intensity Blood Flow Restricted Resistance Exercise on Erythrocyte Oxidative Stress

Acute resistance training with Blood Flow Restriction (BFR) has demonstrated similar acute changes to traditional high-intensity training methods, specifically in muscle size, fatigue, and the hypotensive response during recovery [1-5]. It has been demonstrated resistance training with BFR can elicit similar effects in strength and neuromuscular control at a much lower intensity then traditional resistance exercise. A single bout of low-intensity resistance exercise with BFR has been shown to stimulate muscle protein synthesis, and to enhance both the mammalian target of rapamycin complex 1 (mTORC1) and mitogen-activated protein kinase (MAPK) signaling pathways, which are important metabolic pathways for increasing muscle size and function [6].

Blood Flow Restriction using elastic knee wraps is a practical method of achieving the beneficial results of BFR training without the use of expensive equipment, such as pneumatic tourniquets [7-11]. Practical BFR (pBFR) has been shown to cause venous, but not arterial, occlusion, which can increase metabolic stress with resistance exercise [10]. This version of BFR is much more likely to be utilized in a variety of settings because of its low cost and ease of implementation in a group setting.

One of the suggested pathways for the beneficial effects of BFR training is increased production of Reactive Oxidative Species (ROS) [12, 13]. When there is an imbalance between an individual's ROS production and their antioxidant defenses, they

are said to be in a state of oxidative stress [14]. There are a multitude of factors that can potentially affect an individual's levels of oxidative stress. The oxidative stress response to exercise is increased in men compared to women [15, 16], but the type of sport participation does not affect the oxidative stress response [17]. Conversely, training status does affect this response; trained individuals demonstrate reduced oxidative stress responses and increased antioxidant levels compared to untrained individuals [15, 18, 19]. Over-trained individuals display impaired antioxidant levels [20], and over-training blunted the increase in antioxidant supplementation levels seen acutely or with training [21, 22]. Anti-inflammatory properties of creatine supplementation had no influence on antioxidant levels [23], while cold temperatures revealed decreases in antioxidant defense [24]. Therefore, it is postulated that the intensity of the exercise, rather than the type of exercise, is responsible for increased oxidative stress with a single bout of exercise [25, 26].

There are several studies with conflicting findings on exercise causing oxidative stress; some studies reported that oxidative stress increased following maximal aerobic exercise [20, 27] while others indicated no changes [28, 29], and, one study reported a rise in antioxidants, but not oxidative stress [30].

Anaerobic exercise increases oxidative stress more consistently, as revealed via the Wingate Anaerobic Test [31-33] and resistance training [34-40]. These showed increases in oxidative stress following a single bout of exercise. Isometric and eccentric resistance exercises have also revealed increased oxidative stress [22, 41]. One potential reason for the differences between findings in this line of research was the multitude of time points and markers utilized when investigating oxidative stress. Since

the ROS are transient because of their high reactivity, it is difficult to measure them directly; instead, research has measured their effects on other substances (proteins, lipids, DNA) or the subsequent increase in antioxidants to counteract. Additionally, changes in these substances peak at different time points post-exercise (some up to 24 hours), indicating that some studies may have missed significant perturbations [42, 43].

Oxidative stress has been shown to increase following a single session of the back squat exercise, in which the level of oxidative stress that occurs (specifically in lipid peroxidation) appears to be related to rate of tissue reoxygenation (time taken to replenish oxygen stores in muscle tissue) [39, 40]. Therefore, it is possible that low-intensity exercise with BFR could potentially increase the acute oxidative stress response more than the increase seen with traditional resistance training. Similar to the increases in mTORC1 and MAPK signaling with BFR exercise [6], increased ROS production has been shown to increase adenosine monophosphate-activated protein kinase signaling (AMPK, a potent regulator of skeletal muscle metabolism and gene expression), although excessive levels cause oxidative stress and inactivate AMPK [44, 45]. While two previous studies investigating oxidative stress (damage to plasma lipids and proteins, as well as antioxidant status) during resistance exercise with BFR have shown mixed results [46, 47], neither study utilized a multi-joint exercise such as the back squat and the studies analyzed oxidative stress at different time points.

Takarada et al. compared two low-intensity protocols (one with BFR) and found no differences in oxidative stress, while Goldfarb et al. appeared to achieve mixed results, with high intensity and control protocols (BFR only) achieving increased oxidative stress [46, 47]. These discrepancies in oxidative stress between studies were

likely due to the differences in protocols. Both study protocols used repetition-to-failure protocols, but Takarada et al. utilized knee extensions, while Goldfarb et al. utilized bicep curls and plantarflexion exercises. A comparison between volume-matched protocols utilizing a multi-joint exercise (such as the back squat, which utilizes a much greater muscle mass than the two previous studies) has yet to be made. However, as a recent BFR review article by Pope et al. [13] stated, the topic of oxidative stress "is obviously complicated, and there remain large gaps in our knowledge base at this time."

Petibois and Déléris have previously used Fourier Transformed Infrared Spectrophotometry (FTIR) to analyze the effects of oxidative stress on erythrocytes with acute aerobic exercise and training [48-50]. They reported increased phospholipid peroxidation in the erythrocyte membrane, as well as increased hemoglobin and spectrin denaturation, with aerobic exercise [48, 50]. Khaustova et al. also utilized FTIR to analyze cortisol levels in saliva [51, 52], which has been shown to correlate strongly with serum cortisol levels [53]. Serum cortisol (a common marker for physiological stress) has been shown to increase acutely with low-intensity resistance training with BFR [54]. Thus, assessment of saliva and blood (plasma and erythrocytes) using FTIR has been shown to be a valid method of measuring several markers of interest for physical exercise. This instrument appears to be useful in inexpensively measuring multiple substances in bodily fluids (saliva, blood, plasma, etc.) without requiring a venous blood draw, and can be used to accurately measure oxidative stress in erythrocytes and cortisol in saliva [48-52, 55-57]. To date, no study has utilized FTIR to examine oxidative stress and salivary cortisol levels in erythrocytes with resistance training.

Therefore, the purpose of this study was twofold: 1) to compare the levels of oxidative stress in erythrocytes and salivary cortisol in response to a single session of low-intensity resistance training with BFR or traditional resistance exercise, and 2) compare the differences in the oxidative stress response between trained and untrained individuals. It was hypothesized that both protocols will increase oxidative stress, but that the traditional resistance training will increase it further than the low-intensity BFR training. It was also hypothesized that untrained individuals will have a greater oxidative stress response compared to trained individuals.

Introduction 2: Effects of Short-Term Low-Intensity Blood Flow Restriction Training on Erythrocyte Oxidative Stress

Interestingly, the initial strength gains seen with BFR training appear to develop from hypertrophy, as opposed to the neural adaptations seen with traditional training methods [58]. However, these increases in strength are less than those attained with traditional high-intensity resistance training [59] although similar increases in peripheral blood circulation and vascular endothelial function are seen with both methods of training [60]. Of note is that there were similar adaptations with resistance training with BFR to those seen with hypoxic training, such as increased translocation of glucose transporter type 4 (GLUT-4) transporters to the muscle cell wall [61]. Low-intensity resistance exercise with BFR appears to be an effective method for increasing strength and hypertrophy. Training with pBFR has also been shown to cause similar hypertrophy gains as volume matched traditional resistance training and increase strength gains when used alone or as a supplemental training program [8, 9, 11]. This version of BFR (using elastic knee wraps) is much more likely to be utilized in a variety of settings due to its low cost and ease of implementation in a group setting.

There are several studies with conflicting findings on the effects of exercise on oxidative stress. Some studies report oxidative stress increased following maximal aerobic exercise [20, 27], other studies reporting that it was unchanged [28, 29], and another found that there was a rise in antioxidants -- but not oxidative stress [30]. Mixed aerobic and anaerobic training, such as that for Muay Thai, increased oxidative stress during training and competition [62]. The evidence for oxidative stress with anaerobic exercise is more consistent, with the Wingate Anaerobic Test [31-33] and

resistance training [34-40] shown to increase oxidative stress following a single bout of exercise. Isometric and eccentric resistance exercise has also been shown to increase oxidative stress [22, 41]. Training blunted this response and increased antioxidants in both circuit training [34, 63, 64] and traditional resistance training [65], but the response in the Wingate Anaerobic Test was conflicted [32, 42]. Although two previous studies investigating oxidative stress with BFR have shown mixed results [46, 47], neither utilized a training intervention to examine if BFR training modified the oxidative stress response to exercise.

Petibois and Déléris have previously used Fourier Transformed Infrared Spectrophotometry (FTIR) to analyze the effects of oxidative stress on erythrocytes with acute aerobic exercise and training [48-50]. They reported that aerobic training reduced the lactate response and phospholipid peroxidation in erythrocytes [49]. Khaustova et al. have also utilized FTIR to analyze cortisol levels in saliva [51, 52], which has been shown to correlate strongly with serum cortisol levels [53]. To date, no study has investigated the effects of short-term low-intensity resistance training with BFR on oxidative stress and salivary cortisol levels in erythrocytes utilizing FTIR.

Therefore, the purpose of this study was to examine the effects of a short-term (two weeks) low-intensity BFR resistance training protocol on erythrocyte oxidative stress and salivary cortisol in trained and untrained individuals. It is hypothesized that the training intervention will lessen the acute increase in oxidative stress following exercise in untrained individuals, but there will be no change in the response in trained individuals, and that the cortisol response to exercise will remain unchanged.

Introduction 3: Investigation of Post-Exercise Oxygen Consumption Following a Session of Low-Intensity Blood Flow Restriction Resistance Exercise

Blood Flow Restriction has been used with treadmill walking in order to amplify the intensity of the exercise without increasing the speed or incline of the treadmill. Blood flow restriction has also been shown to increase muscular strength and hypertrophy in response to walking compared to controls [66, 67]. Blood Flow Restriction with walking increases the net metabolic cost (increased oxygen uptake) and relative intensity of the exercise [68] and causes increased minute ventilation during exercise. Additionally, there is an increase in excess post-exercise oxygen consumption (EPOC) following exercise that is partially due to an increase in cumulative oxygen debt [68, 69]. Oxygen consumption during resistance training is often less than that of an aerobic activity of similar intensity [70]. However, the EPOC following resistance exercise is often greater than that of aerobic activity [71], especially with activities that utilize a larger muscle mass, such as the back squat [72, 73]. A review of the literature has failed to reveal a study investigating how low-intensity resistance training with BFR affects EPOC following exercise.

Therefore, the purpose of this study is to examine the EPOC following a single session of low-intensity exercise with BFR and compare it to the EPOC following a session of traditional resistance exercise. It is hypothesized that the EPOC following low-intensity resistance training with BFR will be greater than that of traditional resistance exercise due to the increased number of repetitions and shorter rest periods between sets.

Methods

Study Design

This two-group repeated-measures design was used to assess the acute effects of a single bout of either low-intensity BFR or traditional squat training before and after a short-term (2 week) intervention. Independent measures were the condition (BFR vs. traditional squat), training level (untrained vs. trained), and time (pre- vs. post-intervention and before and after an exercise session). Dependent variables were measures of oxidative stress in erythrocytes as measured with FTIR (phospholipid bilayer peroxidation and hemoglobin unfolding), plasma glucose, plasma and erythrocyte lactate, salivary cortisol, and Borg's Ratings of Perceived Exertion (RPE).

Population

Participants were young males between the ages of 18 and 35 years in an untrained (no regular resistance training within the past three months) (n=11) or trained (regular resistance training over the past three months) (n=8) state. Participants had no absolute or relative contraindications to resistance training and testing, according to the American College of Sports Medicine (ACSM) [74] (Appendix B), as well as no contraindications for BFR exercise (diagnosed hypertension, arrhythmia, ischemic changes in the heart, diabetes, or a BMI greater than 30 $kg \cdot m^{-2}$) [75, 76]. Only individuals classified as low risk by the ACSM were included in the study [74].

Study Visits

Participants completed three study visits before and two visits after a short-term BFR training. These visits consisted of a 3RM squat conducted as part of the initial

visit, and then two acute sessions utilizing either a traditional strength training or BFR training squat protocol prior to training and a final BFR training squat protocol and a 3RM squat following training (see Figure 1). The Initial visit and the two Pre-Training Testing sessions were performed one week after the previous visit. Following the exercise intervention, the order was reversed, with the two acute training sessions preceding a final visit where a 3RM squat was performed (as described below).

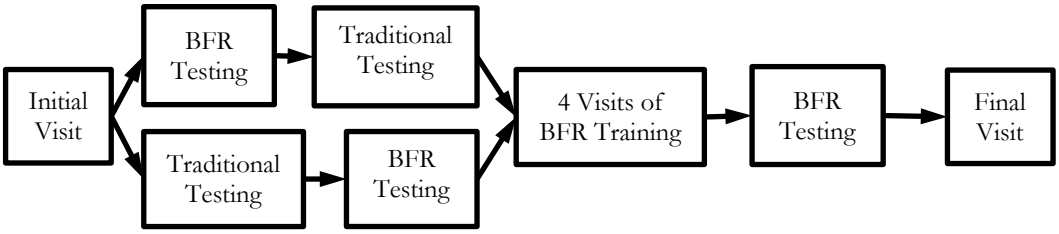

Figure 1. Study Visits

Initial Visit

Participants had an orientation visit prior to the start of testing, during which they completed an informed consent approved by the university's Human Studies Program (Appendix A), as well as a medical history (Appendix C), the AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire (Appendix D) and the George Non-Exercise Test Questionnaire (Appendix E) [74, 77, 78]. Anthropometrics were also collected during this visit, consisting of height (Ht), body mass (BM), blood pressure (BP), thigh girth, and body composition as assessed by skinfold measurements. Participants were also familiarized with the proper form for barbell squat (if needed) and 3RM max testing was conducted.

Final Visit

Participants underwent a final round of anthropometric measurement (Ht, BM, BP, thigh girth, and body composition as assessed by skinfold measurements) during the final visit. A 3RM squat was then performed.

Anthropometric Testing

Height was assessed using a wall mounted stadiometer (Seca 222, Chino, CA) and measured to the 0.1 cm. Body mass was assessed using a certifier balance beam scale (Detecto Model 442, Webb City, MI), measured to quarter pound increments and converted to kilograms. Thigh girth was assessed using a Gulick tape measure, according to the methods described by Lohman et al. [79].

Skinfold thickness was assessed using a 3-site (chest, abdomen, and thigh) measurement (Lange Instruments, Santa Cruz, CA) and body fat percentage was calculated using the Jackson and Pollock formula and the Brozek equation [80, 81]. Measurements were performed on the right side of the body in alternating order to allow for interstitial fluid to return to the tissue and to control for researcher bias. All measurements were performed at least twice; if the results differed by two millimeters or more, subjects were measured a third time, with the mean of the measurements used for analysis.

3RM Squat Testing

Estimated 1RM from low repetition (3 to 5 repetitions) testing has been shown to be highly correlated (r=.99) with actual 1RM for lower extremity strength testing [82]. If needed, participants were instructed in the proper technique for the back squat prior to 3RM testing. Participants completed one warm-up set of 5 to 10 repetitions with a self-

selected light to moderate load, followed by another warm-up set of 5 repetitions with a self-selected moderate load. The load was then increased and the participant was instructed to perform 3 repetitions. If the participant completed the lift, they rested for four minutes before another attempt was made with increased weight [83]. The participant continued this process until they were unable to complete a lift, at which point their last completed lift was used to estimate their 1RM using the National Strength and Conditioning Association 1RM Table (Appendix F) [83].

Testing Sessions

Participants were asked to avoid resistance training, non-steroidal anti-inflammatory medications, alcohol, tobacco, supplements and other medication for 3 days before the testing sessions. Both testing sessions were designed to be volume matched using the volume load method (repetitions*weight, both protocols within 1%) [84]. Blood Pressure was measured at the start of each session using an automated blood pressure cuff following five minutes of seated rest (Model HEM-907XL, Omrom Healthcare Europe B.V., Netherlands). Participants were measured on their right arm whilst seated with their feet planted on the floor and their arm supported. The proper cuff size was used for each participant to avoid incorrect measurements [74].

Traditional Squats

For the traditional squat exercise, participants performed a self-selected warm-up prior to performing their initial squat set. Participants then rested three to five minutes prior to their squat exercise, which consisted of four sets of eight repetitions at 70% of estimated 1RM with 3 to 5 minutes in between sets [74]. Borg's RPE were collected

before and after each set of squats [85]. Estimated total time for this portion of is approximately an hour including EPOC data collection.

Low-Intensity Blood Flow Restriction

Participants performed a self-selected warm-up prior to performing their initial squat set. Blood Flow Restriction was achieved through the use of elastic knee wraps (78 inch, Harbinger Fitness, Durham, North Carolina), which have been shown to occlude venous, but not arterial, blood flow [8-10, 86]. The elastic wraps were applied at the most proximal portion of the thigh at a perceived pressure of 7 out of 10 (tight, but not painful) [8-10, 86]. To ensure that arterial occlusion had not occurred, pulse oximetry (Oxi-Go, Oximeter Plus, Roslyn, New York) was measured at the toes prior to each set and confirmed by palpation. If a pulse was not detectable, the wrap was loosened until it was detectable. Participants then performed 4 sets of squats at 30% of estimated 1RM [86, 87]. The first set consisted of 30 reps, followed by three sets of 15 reps [86, 87]. There were 90 seconds of rest between sets [9, 86, 87]. Borg's Ratings of Perceived Exertion (RPE) were collected before and after each set of squats (Appendix G). Estimated total time for this session was approximately one hour with EPOC data collection.

Metabolic Data

Metabolic data (oxygen consumption, carbon dioxide production, ventilation, heart rate) were collected on a TrueOne 2400 metabolic cart (Parvomedics, Sandy, UT) during the first exercise testing sessions. Participants were fitted with a Hans Rudolph V2 mask (Shawnee, KS) attached to a one-way non-rebreathing valve to collect expired air. Five minutes of resting data were collected prior to exercise; data were also

collected during exercise and for 20 minutes following exercise. The amount and duration of excessive post-exercise oxygen consumption (EPOC) was determined following exercise. The participant's maximal oxygen consumption was estimated using the George non-exercise test [78].

Exercise Intervention

Participants performed six exercise sessions over the course of two to three weeks, with each session separated by at least one day. The exercise intervention will be the same exercise protocol as the Low-intensity BFR testing visit (30% of estimated 1RM for one set of 30 repetitions, followed by 3 sets of 15 repetitions, with 45 seconds between sets) with elastic knee wraps used to create practical BFR. Assessments were performed before and after the last day of the exercise intervention. Participants completed a 5-minute self-selected warm-up prior to exercise. Following the last exercise testing session, another 3RM was performed within one week.

Blood Collection

The participants warmed their hand in front of a portable space heater for roughly two minutes prior to the blood collection. Approximately 200 microliters (about 4 drops) of arterialized blood was collected from a free-flowing digit puncture and collected into a microtainer gel barrier collection tube (365985, Becton-Dickinson, Franklin Lakes, New Jersey). Blood collection was completed at rest (prior to exercise) as well as immediately post-exercise, 30 minutes post exercise, and 24 hours post-exercise.

Blood samples were immediately centrifuged for 10 minutes at 1,500 g. Supernatant plasma was then removed and either analyzed or stored at -20°C for analysis within 24 hours.[48-50, 55-57] Erythrocytes were then removed from the

microtainer and washed with a saline solution before being spun again at 1,000 g for 30 minutes. This process was repeated two additional times to remove the buffy layer on the cells before the erythrocytes were either stored at -20°C for analysis within 24 hours or analyzed immediately [48-50, 55].

Plasma samples were defrosted if needed and 20 microliters of plasma was diluted with 80 microliters of water (1:4 ratio) before being homogenized with a vortex mixer for 10 seconds. A 35-microliter sample of the solution was then placed on a zinc-selenide disc and placed in a vacuum drying chamber until the liquid in the sample had sublimated. A similar process was utilized for erythrocytes, with exception of the amount of water added to the sample. In the case of erythrocytes, 10 microliters of packed cells were diluted with 190 microliters of water (1:19 ratio) and homogenized with an agitator for 10 seconds. Spectra were then collected using a Nicolet IS-5 infrared spectrophotometer (Thermo-Scientific, Waltham, Massachusetts) with a KBr beam splitter at a resolution of 2.0 cm^{-1} resolution for 32 scans between 500 and 4000 cm^{-1} [48-50, 55].

Saliva Collection

Saliva was collected prior to, immediately after, and 30 minutes after exercise for analysis of salivary cortisol. Participants were instructed to ingest nothing by mouth for at least one hour prior to saliva collection. Participants chewed on cotton balls for several minutes to collect saliva. Once the cotton balls were saturated, they were placed in a 20-milliliter syringe; the plunger of the syringe was then replaced and depressed to move the saliva into a 6-milliliter cryovial. This method of saliva collection has been found to be better correlated and a better predictor of serum cortisol level

when compared to a drool collection method (r=0.813 vs. r=0.735) [53]. The saliva was then placed in a centrifuge and spun at 1000 g for 10 minutes to remove particulate matter. Two microliters of saliva were then placed on the spectrophotometer and allowed to dry prior to spectral analysis.

Spectra were recorded using a Nicolet IS-5 infrared spectrophotometer (Thermo-Scientific, Waltham, Massachusetts), which fitted with an attenuated total reflectance accessory with a diamond disc, at a resolution of 4.0 cm^{-1} resolution for 32 scans between 4000 and 600 cm^{-1} [51, 52]. All samples were measured in triplicate.

Sample Analysis

Plasma

Correction factors for glucose and lactate concentrations for plasma volume shift were determined using change in total spectral area (4000 to 500 cm^{-1}) as determined by the equation: Exercise Plasma spectrum – 1.14 * Resting Exercise spectrum. Thus, if plasma volume changed by *n*%, then the glucose and lactate values were adjusted by *n*% [88]. For measurement of blood glucose concentration in plasma, spectral area from 1062-997 cm^{-1} was integrated and multiplied by 7.27 $mmol \cdot U^{-1}$ and then adjusted for plasma volume shift if needed [56, 57]. Glucose spectra were subtracted prior to measurement of lactate, then the spectral area from 1163-1107 cm^{-1} was integrated, multiplied by 3.67 $mmol \cdot L^{-1} \cdot U^{-1}$ and corrected for plasma volume shift as needed [56].

Erythrocytes

Optical density of spectra and variation of total spectral area were assessed to determine the validity of the sample. Spectra were assessed in the regions shown in

Table 1 [48-50, 55]. Petibois and Déléris reported that increased oxidative stress from aerobic exercise resulted in the following changes [48-50, 55]:

- Decrease in $v_{as}(CH_2)$
- Increase in v(=CH)
- Decrease in the CH_2/CH_3 ratio
- Decrease in v(P=O)
- Increase in v(C=O)
- Increase in v(C-O)
- Decrease in δ(N-H)
- Increase in the v(C=O)/δ(N-H) ratio
- No changes in $v_{as}(CH_3)$

Table 1. Major Molecular Bands for Erythrocyte Spectra

Spectral Intervals (cm^{-1})	IR Bands	Erythrocyte
3020-3000	v(=CH)	(olefinic) unsaturated fatty acids, cholesterol esters
2990-2950	$v_{as}(CH_3)$	(methyl) phospholipids, cholesterol esters, fatty acids
2950-2880	$v_{as}(CH_2)$	(methylene) phospholipids, long chain fatty acids
2880-2860	$v_s(CH_3)$	(methyl) phospholipids, fatty acids
2870-2830	$v_s(CH_2)$	(methylene) phospholipids, long chain fatty acids
1739-1713	v(C=O)	Phospholipids, cholesterol esters, glycerides
1713-1589	v(C=O)	(amide I) β-sheets: proteins, turns, coils
1589-1474	δ(N-H)	(amide II) α-helix: proteins
1480-1430	$\delta_{as}(CH_3)$, $\delta_{as}(CH_2)$, $\delta_s(CH_3)$, $\delta_s(CH_2)$	phospholipids, fatty acids, glycerides
1420-1370	$v(COO^-)$	(carboxylate ions) amino acids
1257-1201	v(P=O)	(phosphate) phospholipids
1300-900	v(C-O)	Polysaccharides, glucose, lactate

v: stretching vibrations; δ: bending (scissoring) vibrations; *s*: symmetric; *as*: asymmetric

Since the phospholipid bilayer is the only cellular structure in erythrocytes that contains fatty acyl chains (ν(=CH), $\nu_{as}(CH_3)$, $\nu_{as}(CH_2)$, and ν(P=O)), it can be assumed that changes to these absorbencies are reflective of changes in the membrane phospholipids. Likewise, changes in the ν(C-O) region can be attributed to lactate, and changes in the ν(C=O) (amide I) and δ(N-H) regions can be attributed to proteins since they are the only substances with those bonds. Furthermore, the protein changes are most likely in response to hemoglobin denaturation since it comprises 90% of the protein in the erythrocyte [50].

Saliva

Spectral areas were measured from 1943-1526 cm^{-1}, 1391-1249 cm^{-1}, and 1115-973 cm^{-1} and used to determine salivary cortisol levels. Prior to the study, partial least squares analysis was used to create a regression curve for analysis using the methods described by Khaustova et al. [51, 52].

Statistics

Statistics were performed using SPSS version 24.0 (IBM, Armonk, New York) with a significance level set at $p<0.05$. Sample size was determined using G*Power 3.1 (Universität Kiel, Kiel, Germany) using effect sizes from the literature. Normality was assessed using visual inspection, which was further confirmed with Shapiro-Wilks tests. Correlations between variables were assessed using Pearson's product moment correlation coefficient. Differences in the acute oxidative stress response within and between groups (trained vs. untrained) at each time point (pre- and post-training) were assessed using a three-way, mixed repeated measures analysis of variance (ANOVA). Differences between the EPOC for the two training protocols and between groups was

assessed using a two-by-two repeated measures ANOVA. Post hoc analyses were performed using Fisher's Least Significant Difference Test.

Results

Participants

Of the 19 enrolled participants, three participants withdrew or were removed from the study following the initial study visits and did not complete the training portion of the protocol, while an additional two are currently completing the training protocol. These participants were all from the trained wing of the study, while all eight untrained participants completed the study. Participants had a mean age of 24.6±1.3 years. The overall mean height was 1.77±0.06 meters and overall mean body mass was 77.2±10.7 kg, resulting in a BMI of 24.8±3.3. Overall estimated maximal oxygen consumption from the George non-exercise questionnaire was 50.2±4.6 $mL \cdot kg^{-1} \cdot min^{-1}$. Data for the trained and untrained groups can be seen in Table 2.

Table 2. Demographic Data

	Overall	Trained	Untrained
Enrolled n	19	11	8
Completed n	14	6	8
Age	24.6±1.3	23.6±1.1	26.3±3.0
Height (cm)	176.5±6.4	176.3±8.4	176.9±5.6
Body Mass (kg)	77.2±10.7	78.5±8.9	73.0±12.1
Body Mass Index	24.8±3.3	25.2±1.8	23.4±4.2
Estimated VO_{2MAX} ($mL \cdot kg^{-1} \cdot min^{-1}$)	50.2±4.6	50.3±2.8	50.3±5.7

cm: centimeters; kg: kilograms; mL: milliliters; min: minute

Anthropometrics

There were no significant differences between the trained and untrained groups in height, BM, BMI, BF%, SBP, DBP, and resting HR (p>0.05 for all). However, the proximal and mid-thigh girth measurements were significantly greater in the trained group compared to the untrained group (p=0.036 and p=0.020 respectively), while the distal thigh girth was insignificant (p=0.139). Additionally, the 3RM back squat was significantly lower in the untrained compared to trained group (272.5±72.8 vs. 186.3±52.8 lbs, p=0.04). Overall and individual means and standard deviations for each group can be seen in Table 3.

Table 3. Anthropometric Data at Entry

	Overall	Trained	Untrained
Body Mass (kg)	77.2±10.7	78.5±8.9	73.0±12.1
BMI	24.8±3.3	25.2±1.8	23.4±4.2
BF %	13.05±4.17%	14.11±4.76%	12.46±4.05%
SBP (mmHg)	122.5±8.5	123.4±10.7	121.8±3.6
DBP (mmHg)	73.5±6.8	73.5±9.6	72.6±5.1
Resting HR (BPM)	69.3±12.2	70.5±10.9	69.0±15.3
Distal Thigh Girth (cm)	38.90±2.49	39.65±2.33	37.93±2.82
Mid-Thigh Girth (cm)	56.53±5.09	58.19±4.39*	53.52±5.27
Proximal Thigh Girth (cm)	59.49±4.70	61.26±4.16*	56.96±5.14
3RM Back Squat (lbs)	243.95±77.52	272.50±72.80*	186.25±52.83
30% of Estimated 1RM	79.68±24.87	89.00±24.01*	61.25±16.42
70% of Estimated 1RM	185.42±58.44	207.50±55.87*	141.88±38.91

kg: kilograms; BMI: Body Mass Index; BF %: Body Fat Percentage; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; mmHg: millimeters of mercury; HR: Heart Rate; BPM: Beats Per Minute; cm: centimeters; 3RM: 3 repetition maximum; 1RM: 1 repetition maximum; lbs: pounds; * significantly different from Untrained group at the p=0.05 level

Spectral Values

The mean spectral value for the peak at 1654 cm^{-1} was 1.454±0.546 with a variance of 0.018 in plasma and 1.810±0.579 with a variance of 0.030 in erythrocytes. The mean values at 4000 cm^{-1} were 0.012±0.023 with a variance of 0.001 in plasma and 0.006±0.014 with a variance of 0.001 in erythrocytes. The mean values at 2100 cm^{-1} were 0.043±0.026 with a variance of 0.001 in plasma and 0.053±0.018 with a variance of 0.002 in erythrocytes.

Comparison of Low-Intensity Back Squats with BFR to Traditional High-Intensity Back Squats

Plasma Values

Mean values and standard deviations for plasma total spectral area, glucose and lactate values, both overall and for each group, can be seen in Table 4. There were no significant main effects between testing sessions, over time during each testing session, or between training statuses as well as no significant interaction effects between them ($p>0.05$). There were also no significant main effects or interaction effects for plasma glucose. The only significant main effect for time was present in plasma lactate; all other main effects and interactions were nonsignificant. The Post-1 (immediately post exercise) time point was significantly elevated compared to pre-exercise (Pre) resting time point (Mean Difference 0.358, $p=0.006$), 30-minute post-exercise (Post-2) time point (Mean Difference 0.309, $p=0.012$), and 24-hour post-exercise (24 Post) time point (Mean Difference 0.433, $p=0.013$). Alpha levels, effect sizes, and statistical powers for comparisons in plasma values between low-intensity squats with BFR and traditional high intensity squats are shown in Table 5.

Table 4. Comparison of Plasma Values Between Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Low-Intensity Squats with BFR				Traditional High-Intensity Squats			
		Pre	Post-1	Post-2	24 Post	Pre	Post-1	Post-2	24 Post
Total Spectral Area	Overall	926.27 ±423.32	884.61 ±383.01	930.83 ±259.29	1028.44 ±489.47	960.44 ±262.20	945.31 ±284.54	953.28 ±345.46	806.50 ±293.86
	Trained	910.94 ±454.81	769.37 ±356.31	900.03 ±222.98	1088.04 ±576.70	921.67 ±288.34	960.94 ±248.02	973.85 ±347.36	741.34 ±275.62
	Untrained	945.43 ±410.47	1028.66 ±387.75	965.49 ±307.01	953.93 ±377.89	1008.91 ±235.03	925.78 ±341.70	927.56 ±365.16	890.28 ±316.28
Glucose	Overall	64.68 ±37.50	64.60 ±48.56	61.71 ±31.19	68.95 ±44.77	68.54 ±29.71	71.09 ±28.56	64.56 ±25.56	113.54 ±199.48
	Trained	60.61 ±42.99	47.23 ±42.95	58.51 ±37.88	63.66 ±52.55	58.22 ±25.81	74.42 ±27.27	61.24 ±17.35	56.84 ±20.62
	Untrained	69.76 ±31.40	86.32 ±48.85	65.30 ±23.55	75.56 ±35.02	81.44 ±30.73	66.93 ±31.46	68.72 ±34.12	186.44 ±296.47
Lactate	Overall	1.22 ±0.81	1.47 ±0.84	1.38 ±0.62	1.29 ±0.58	1.28 ±0.54	1.76 ±0.75	1.30 ±0.57	1.16 ±0.59
	Trained	1.25 ±0.95	1.30 ±0.91	1.38 ±0.72	1.33 ±0.89	1.18 ±0.54	1.90 ±0.68	1.32 ±0.50	0.98 ±0.40
	Untrained	1.16 ±0.64	1.68 ±0.75	1.38 ±0.52	1.31 ±0.75	1.42 ±0.54	1.58 ±0.85	1.27 ±0.68	1.38 ±0.75

BFR: blood flow restriction

Table 5. Main Effects, Interaction Effects, Effect Size, and Power for Comparisons of Plasma Values Between Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
Total Spectral Area	*p*	0.608	0.660	0.443	0.133	0.653	0.877	0.438
	η_p^2	0.018	0.034	0.040	0.116	0.014	0.015	0.058
	Power (β)	0.078	0.151	0.114	0.473	0.071	0.090	0.236
Glucose	*p*	0.322	0.317	0.168	0.525	0.549	0.294	0.198
	η_p^2	0.061	0.064	0.116	0.030	0.023	0.070	0.100
	Power (β)	0.161	0.176	0.275	0.108	0.089	0.188	0.268
Lactate	*p*	0.927	**0.015***	0.714	0.228	0.873	0.974	0.121
	η_p^2	0.001	**0.194**	0.009	0.085	0.002	0.005	0.113
	Power (β)	0.051	**0.790**	0.064	0.370	0.053	0.062	0.491

η_p^2: partial eta squared; β: power; *indicates $p<0.05$

Fatty Acid Moieties in the Phospholipid Bilayer

Mean values and standard deviations for markers of the fatty acid moieties in the phospholipid bilayer, both overall and for each group, can be seen in Table 6. There

were no significant main effects in between testing sessions, over time during each testing session, or between training statuses -- as well as no significant interaction effects between these main effects (p>0.05). Alpha levels, effect sizes, and statistical powers for comparisons in plasma values between low-intensity squats with BFR and traditional high intensity squats are shown in Table 7. There appear to be no changes in phospholipid bilayer in erythrocytes due to oxidative stress following a single session of back squats utilizing both a high-intensity hypertrophy protocol or a low-intensity protocol with BFR.

Table 6. Comparison of Fatty Acid Moieties in the Phospholipid Bilayer Between Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Low-Intensity Squats with BFR				Traditional High-Intensity Squats			
		Pre	Post-1	Post-2	24 Post	Pre	Post-1	Post-2	24 Post
V=(CH)	Overall	0.079 ±0.014	0.092 ±0.063	0.119 ±0.115	0.118 ±0.125	0.076 ±0.029	0.091 ±0.049	0.085 ±0.052	0.073 ±0.038
	Trained	0.076 ±0.029	0.083 ±0.048	0.135 ±0.143	0.134 ±0.147	0.077 ±0.034	0.103 ±0.057	0.093 ±0.064	0.079 ±0.044
	Untrained	0.079 ±0.011	0.105 ±0.079	0.102 ±0.079	0.098 ±0.096	0.074 ±0.025	0.076 ±0.034	0.075 ±0.032	0.065 ±0.028
Vas(CH_3)	Overall	2.082 ±0.331	2.315 ±1.614	3.021 ±2.889	3.165 ±3.388	2.042 ±0.769	2.243 ±1.112	2.187 ±1.393	1.905 ±0.948
	Trained	2.061 ±0.394	2.088 ±1.353	3.332 ±3.643	3.657 ±4.023	2.123 ±0.917	2.610 ±1.343	2.460 ±1.769	2.109 ±1.091
	Untrained	2.108 ±0.255	2.597 ±1.952	2.670 ±1.908	2.551 ±2.506	1.942 ±0.580	1.785 ±0.503	1.846 ±0.671	1.644 ±0.718
Vas(CH_2)	Overall	9.982 ±1.531	11.437 ±8.116	14.338 ±13.849	14.984 ±16.057	9.837 ±3.790	10.773 ±5.471	10.366 ±6.482	9.018 ±4.394
	Trained	9.825 ±1.889	10.049 ±6.311	15.903 ±17.563	17.340 ±19.037	10.083 ±4.453	12.399 ±6.673	11.546 ±8.227	9.908 ±4.995
	Untrained	10.179 ±1.011	13.173 ±10.127	12.577 ±8.896	12.039 ±11.933	9.529 ±3.032	8.74 ±2.631	8.892 ±3.247	7.874 ±3.504
Vas(CH_2) : Vas(CH_3) Ratio	Overall	4.803 ±0.224	5.371 ±3.604	6439 ±6.614	6.605 ±6.884	4.815 ±0.325	5.121 ±1.971	5.123 ±1.956	4.264 ±1.392
	Trained	4.769 ±0.126	4.468 ±2.503	7.062 ±8.695	6.605 ±6.884	4.764 ±0.162	5.611 ±2.320	5.593 ±2.412	4.350 ±1.696
	Untrained	4.845 ±0.312	6.500 ±4.569	5.739 ±3.537	7.192 ±8.529	4.815 ±0.325	5.121 ±1.971	5.123 ±1.956	4.264 ±1.392
V=(CH) : Vas(CH_3) Ratio	Overall	0.038 ±0.002	0.041 ±0.005	0.040 ±0.006	0.038 ±0.005	0.038 ±0.006	0.040 ±0.006	0.039 ±0.005	0.038 ±0.006
	Trained	0.038 ±0.002	0.041 ±0.005	0.042 ±0.006	0.037 ±0.005	0.038 ±0.006	0.039 ±0.004	0.038 ±0.004	0.037 ±0.006
	Untrained	0.037 ±0.002	0.040 ±0.006	0.037 ±0.004	0.038 ±0.004	0.038 ±0.005	0.041 ±0.009	0.039 ±0.007	0.040 ±0.005
V(P=O)	Overall	1.555 ±0.280	1.736 ±1.054	2.285 ±2.494	2.251 ±2.403	1.491 ±0.599	1.726 ±0.962	1.583 ±0.962	1.397 ±0.690
	Trained	1.557 ±0.337	1.598 ±0.886	2.689 ±3.263	2.544 ±2.831	1.510 ±0.678	1.950 ±1.168	1.735 ±1.200	1.538 ±0.789
	Untrained	1.553 ±0.209	1.908 ±1.276	1.831 ±1.269	1.885 ±1.854	1.467 ±0.529	1.446 ±0.578	1.392 ±0.566	1.216 ±0.541
RBC Glucose	Overall	0.196 ±0.048	0.227 ±0.151	0.299 ±0.344	0.295 ±0.316	0.187 ±0.078	0.227 ±0.134	0.202 ±0.124	0.175 ±0.089
	Trained	0.203 ±0.057	0.211 ±0.116	0.358 ±0.456	0.336 ±0.370	0.188 ±0.087	0.251 ±0.156	0.219 ±0.154	0.193 ±0.099
	Untrained	0.187 ±0.034	0.246 ±0.192	0.232 ±0.152	0.244 ±0.248	0.186 ±0.072	0.196 ±0.100	0.181 ±0.079	0.152 ±0.074
RBC Lactate	Overall	0.236 ±0.044	0.288 ±0.228	0.350 ±0.360	0.369 ±0.368	0.227 ±0.090	0.261 ±0.149	0.233 ±0.145	0.206 ±0.101
	Trained	0.240 ±0.055	0.255 ±0.150	0.404 ±0.469	0.422 ±0.411	0.226 ±0.100	0.291 ±0.180	0.229 ±0.112	0.229 ±0.112
	Untrained	0.231 ±0.026	0.329 ±0.307	0.290 ±0.190	0.302 ±0.320	0.230 ±0.083	0.224 ±0.099	0.205 ±0.087	0.176 ±0.084

v: stretching vibrations; *s*: symmetric; *as*: asymmetric; RBC: Red Blood Cell

Table 7. Main Effects, Interaction Effects, Effect Size, and Power for Comparisons of Fatty Acid Moieties in the Phospholipid Bilayer Between Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
V=(CH)	*p*	0.280	0.462	0.489	0.183	0.850	0.662	0.469
	η_p^2	0.073	0.043	0.030	0.101	0.002	0.021	0.046
	Power (β)	0.183	0.147	0.102	0.342	0.054	0.094	0.167
Vas(CH_3)	*p*	0.276	0.545	0.408	0.155	0.774	0.613	0.442
	η_p^2	0.074	0.032	0.043	0.112	0.005	0.025	0.049
	Power (β)	0.186	0.118	0.126	0.366	0.059	0.102	0.171
Vas(CH_2)	*p*	0.271	0.595	0.470	0.166	0.790	0.573	0.392
	η_p^2	0.075	0.027	0.033	0.107	0.005	0.029	0.056
	Power (β)	0.189	0.108	0.107	0.355	0.057	0.113	0.194
Vas(CH_2) : Vas(CH_3) Ratio	*p*	0.331	0.584	0.794	0.767	0.767	0.552	0.403
	η_p^2	0.059	0.028	0.004	0.006	0.006	0.032	0.052
	Power (β)	0.157	0.111	0.057	0.059	0.059	0.119	0.172
V=(CH) : Vas(CH_3) Ratio	*p*	0.605	0.090	0.808	0.505	0.576	0.949	0.930
	η_p^2	0.017	0.125	0.004	0.041	0.020	0.007	0.004
	Power (β)	0.079	0.543	0.056	0.155	0.084	0.070	0.060
V(P=O)	*p*	0.300	0.585	0.427	0.196	0.300	0.631	0.196
	η_p^2	0.067	0.028	0.040	0.098	0.067	0.023	0.098
	Power (β)	0.172	0.109	0.120	0.314	0.172	0.098	0.314
RBC Glucose	*p*	0.290	0.537	0.419	0.176	0.943	0.650	0.585
	η_p^2	0.070	0.034	0.041	0.104	0.000	0.022	0.031
	Power (β)	0.177	0.123	0.122	0.340	0.051	0.096	0.124
RBC Lactate	*p*	0.200	0.589	0.497	0.099	0.942	0.580	0.520
	η_p^2	0.100	0.031	0.029	0.121	0.000	0.032	0.031
	Power (β)	0.242	0.124	0.100	0.527	0.051	0.126	0.124

v: stretching vibrations; *δ*: bending (scissoring) vibrations; *s*: symmetric; *as*: asymmetric; η_p^2: partial eta squared; β: power; RBC: Red Blood Cell; *indicates *p*<0.05

Proteins in Erythrocytes

Mean values and standard deviations for markers of the fatty acid moieties in the phospholipid bilayer, both overall and for each group, can be seen in Table 8. There were no significant main effects in between testing sessions, over time during each testing session, or between training statuses in all measures (*p*>0.05). There was a significant time-by-training-status interaction in the V(C=O) bond (*p*=0.020). While there

were no differences over time in the Trained group, the Untrained group had significant decreases at the Post-2 (30 minutes after exercise) and 24 Post (24 hours after exercise) compared to the pre-exercise (Pre) time point (p<0.05). A Session by Time by Training Status was also present, with the Post-1 (immediately post-exercise) timepoint being significantly higher during the low-intensity with BFR condition compared to the traditional squats in Untrained, but not Trained individuals, while the Trained individuals had a significantly higher 24 Post value in the low-intensity with BFR condition. There were no other significant interaction effects present (p>0.05). Alpha levels, effect sizes, and statistical powers for comparisons in plasma values between low-intensity squats with BFR and traditional high intensity squats are shown in Table 9.

Table 8. Comparison of Proteins in Erythrocytes Between Low Intensity Squats with BFR and Traditional High Intensity Squats

		Low-Intensity Squats with BFR				Traditional High-Intensity Squats			
		Pre	Post-1	Post-2	24 Post	Pre	Post-1	Post-2	24 Post
V(C=O)	Overall	0.200 ±0.229	0.230 ±0.418	0.167 ±0.191	0.226 ±0.209	0.168 ±0.146	0.102 ±0.063	0.132 ±0.079	0.101 ±0.067
	Trained	0.142 ±0.106	0.076 ±0.066	0.194 ±0.189	0.282 ±0.247	0.153 ±0.085	0.114 ±0.073	0.148 ±0.083	0.108 ±0.082
	Untrained	0.272 ±0.319	0.421 ±0.585	0.136 ±0.202	0.155 ±0.130	0.187 ±0.205	0.087 ±0.048	0.113 ±0.075	0.091 ±0.047
Amide I V(C=O)	Overall	69.777 ±14.864	78.035 ±55.568	98.040 ±74.121	112.392 ±118.336	72.258 ±24.827	77.936 ±31.467	75.629 ±41.431	67.101 ±32.702
	Trained	69.332 ±15.737	70.392 ±54.506	102.354 ±88.512	134.193 ±144.270	76.086 ±28.722	89.780 ±33.536	84.828 ±52.938	74.704 ±38.443
	Untrained	70.333 ±14.749	87.590 ±59.089	93.186 ±59.607	85.140 ±75.631	67.474 ±19.728	63.131 ±22.534	64.132 ±17.137	57.327 ±22.472
Amide II (ō-N)	Overall	38.259 ±6.439	42.420 ±30.280	53.823 ±43.528	60.474 ±65.763	38.668 ±14.796	41.144 ±17.944	41.200 ±25.155	35.403 ±17.786
	Trained	37.752 ±7.135	37.621 ±27.396	56.685 ±51.963	71.367 ±79.790	41.397 ±17.233	48.455 ±20.481	46.782 ±31.835	39.369 ±20.360
	Untrained	38.892 ±5.866	48.418 ±34.462	50.603 ±34.965	46.857 ±44.003	35.257 ±11.219	32.005 ±8.417	34.223 ±11.556	30.305 ±13.572
Amide I : Amide II Ratio	Overall	1.812 ±0.142	1.824 ±0.142	1.850 ±0.142	1.871 ±0.138	1.926 ±0.282	1.912 ±0.277	1.895 ±0.277	1.948 ±0.287
	Trained	1.826 ±0.161	1.815 ±0.147	1.827 ±0.150	1.884 ±0.139	1.872 ±0.127	1.877 ±0.122	1.844 ±0.140	1.912 ±0.126
	Untrained	1.794 ±0.121	1.836 ±0.144	1.875 ±0.138	1.854 ±0.144	1.993 ±0.403	1.955 ±0.404	1.960 ±0.391	1.995 ±0.424

v: stretching vibrations; *δ*: bending (scissoring) vibrations

Table 9. Main Effects, Interaction Effects, Effect Size, and Power for Comparisons of Erythrocyte Proteins Between Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
V(C=O)	*p*	0.104	0.506	0.511	0.287	0.385	**0.020***	**0.047***
	η_p^2	0.157	0.041	0.027	0.075	0.048	**0.222**	**0.181**
	Power (β)	0.367	0.152	0.097	0.247	0.134	**0.720**	**0.584**
Amide I V(C=O)	*p*	0.281	0.525	0.349	0.099	0.734	0.471	0.340
	η_p^2	0.072	0.034	0.055	0.136	0.007	0.041	0.065
	Power (β)	0.183	0.124	0.149	0.457	0.062	0.140	0.224
Amide II (ō-N)	*p*	0.267	0.533	0.363	0.119	0.670	0.545	0.331
	η_p^2	0.076	0.033	0.052	0.127	0.012	0.032	0.066
	Power (β)	0.191	0.119	0.143	0.420	0.067	0.116	0.226
Amide I : Amide II Ratio	*p*	0.471	0.573	0.503	0.162	0.800	0.517	0.684
	η_p^2	0.033	0.030	0.028	0.109	0.004	0.036	0.022
	Power (β)	0.107	0.115	0.099	0.359	0.057	0.130	0.101

v: stretching vibrations; *δ*: bending (scissoring) vibrations; η_p^2: partial eta squared; β: power; *indicates *p*<0.05

Cortisol Salivary Markers

Mean values and standard deviations for markers of salivary cortisol, both overall and for each group, can be seen in Table 10. There were no significant main effects in between testing sessions, over time during each testing session, or between training statuses as well as no significant interaction effects between these main effects (*p*>0.05) with the exception of Session-by-Time interaction for the 3500-3200 cm^{-1} band (*p*=0.032). There were no changes over time during the traditional high-intensity back squat exercise but during the low-intensity back squat session with BFR, the Post-2 timepoint (30 minutes post-exercise) was significantly increased compared to Post-1 timepoint (immediately post-exercise). Alpha levels, effect sizes, and statistical powers for comparisons in cortisol salivary markers between low-intensity squats with BFR and traditional high-intensity squats are shown in Table 11.

Table 10. Comparison of Markers for Salivary Cortisol Between Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Low-Intensity Squats with BFR			Traditional High-Intensity Squats		
		Pre	Post-1	Post-2	Pre	Post-1	Post-2
3500-3200 cm^{-1}	Overall	47.220 ±26.913	38.813 ±20.950	57.036 ±27.989	51.015 ±33.002	44.785 ±25.077	34.693 ±22.927
	Trained	48.374 ±22.449	37.418 ±22.487	50.565 ±28.874	43.637 ±30.871	50.340 ±28.597	34.649 ±23.920
	Untrained	45.778 ±33.269	40.556 ±20.236	63.507 ±27.373	60.238 ±35.280	38.536 ±20.456	34.748 ±23.258
1900-900 cm^{-1}	Overall	21.910 ±8.333	21.600 ±10.135	26.129 ±8.381	21.628 ±13.578	24.199 ±11.612	18.935 ±10.364
	Trained	21.627 ±6.388	19.653 ±9.404	24.899 ±5.525	17.903 ±11.059	25.320 ±12.937	18.645 ±11.519
	Untrained	22.263 ±10.766	24.034 ±11.117	27.360 ±10.794	26.284 ±15.681	22.937 ±10.653	19.298 ±9.486
1946-1526 cm^{-1}	Overall	10.855 ±4.144	10.637 ±4.500	13.028 ±4.160	10.850 ±6.167	12.155 ±5.852	9.410 ±5.002
	Trained	10.856 ±2.974	9.923 ±4.353	11.993 ±2.933	9.436 ±5.697	13.229 ±6.611	9.566 ±5.543
	Untrained	10.852 ±5.508	11.529 ±4.814	14.062 ±5.102	12.617 ±6.650	10.947 ±5.018	9.215 ±4.603
1391-1249 cm^{-1}	Overall	2.612 ±4.085	24.09 ±1.399	3.114 ±1.051	2.535 ±1.784	2.843 ±1.564	2.222 ±1.323
	Trained	2.489 ±0.794	2.162 ±1.314	3.080 ±0.655	1.961 ±1.429	2.823 ±1.526	2.063 ±1.408
	Untrained	2.766 ±1.415	2.717 ±1.530	3.149 ±1.392	3.252 ±2.011	2.865 ±1.711	2.420 ±1.272
1115-973 cm^{-1}	Overall	2.950 ±1.016	3.008 ±1.499	3.442 ±1.096	2.938 ±2.330	3.232 ±1.375	2.641 ±1.444
	Trained	2.938 ±0.898	2.613 ±1.286	3.400 ±0.611	2.264 ±1.171	3.071 ±1.408	2.493 ±1.497
	Untrained	2.966 ±1.211	3.501 ±1.683	3.483 ±1.482	3.781 ±3.156	3.412 ±1.408	2.826 ±1.469

BFR: blood flow restriction; cm: centimeter

Table 11. Main Effects, Interaction Effects, Effect Size, and Power for Salivary Cortisol Markers

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
3500-3200 cm^{-1}	*p*	0.520	0.185	0.985	**0.032***	0.757	0.627	0.594
	η_p^2	0.033	0.122	0.000	**0.232**	0.008	0.035	0.039
	Power (β)	0.094	0.342	0.050	**0.656**	0.060	0.119	0.128
1900-900 cm^{-1}	*p*	0.564	0.903	0.772	0.098	0.934	0.678	0.420
	η_p^2	0.026	0.003	0.007	0.164	0.001	0.021	0.065
	Power (β)	0.085	0.055	0.059	0.463	0.051	0.082	0.188
1946-1526 cm^{-1}	*p*	0.614	0.879	0.920	0.058	0.649	0.723	0.357
	η_p^2	0.020	0.005	0.001	0.196	0.016	0.017	0.076
	Power (β)	0.077	0.057	0.051	0.558	0.072	0.076	0.217
1391-1249 cm^{-1}	*p*	0.637	0.915	0.652	0.146	0.709	0.610	0.688
	η_p^2	0.018	0.003	0.016	0.138	0.011	0.029	0.028
	Power (β)	0.073	0.054	0.071	0.387	0.064	0.095	0.105
1115-973 cm^{-1}	*p*	0.651	0.861	0.492	0.385	0.610	0.576	0.395
	η_p^2	0.016	0.005	0.037	0.071	0.021	0.031	0.069
	Power (β)	0.071	0.057	0.101	0.203	0.077	0.099	0.199

η_p^2: partial eta squared; β: power; cm: centimeter; *indicates $p<0.05$

Excessive Post-Exercise Oxygen Consumption

Mean values and standard deviations for oxygen consumption, both overall and for each group, can be seen in Table 12. There were no significant differences in resting oxygen consumption or peak oxygen consumption during exercise between the low-intensity back squats with BFR and traditional high-intensity back squats overall and between trained vs untrained individuals ($p>0.05$ for main and interaction effects, see Table 13). While there were no significant main effects between conditions as well as Session by Group interactions for either measure of EPOC ($p>0.05$ for all), the trained individuals had a significantly higher magnitude of EPOC (20.90 kcal vs 13.464 kcal, mean difference 7.437 kcals, $p=0.004$) and duration of EPOC (16.43 min vs. 12.317 min, mean difference 4.11 min, $p=0.031$).

Table 12. Comparison of Oxygen Consumption Before, During, and After Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Traditional High-Intensity	Low-Intensity with BFR
Resting VO_2 ($L \cdot min^{-1}$)	Overall	0.330±0.143	0.274±0.070
	Trained	0.391±0.210	0.282±0.057
	Untrained	0.308±0.094	0.267±0.088
EPOC Magnitude (Kcal)	Overall	16.73±7.29	18.07±8.20
	Trained	17.78±4.74	25.04±4.20
	Untrained	12.76±5.89	14.17±6.57
EPOC Duration (Min)	Overall	14.16±5.78	14.82±6.12
	Trained	14.45±6.86	20.15±2.13
	Untrained	13.37±6.11	11.26±5.81
Relative Peak VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	Overall	29.95±4.33	29.01±3.50
	Trained	33.30±3.22	29.85±3.00
	Untrained	28.56±4.43	28.69±4.39

L: liters; min: minute; kcal: kilocalories; VO_2: oxygen consumption; mL: milliliters; kg: kilogram

Table 13. Main Effects, Interaction Effects, Effect Size, and Power for Oxygen Consumption Before, During, and After Low-Intensity Squats with BFR and Traditional High- Intensity Squats

		Session	Training Status	Session * Training Status
Resting VO_2 ($L \cdot min^{-1}$)	*P*	0.084	0.566	0.624
	η_p^2	0.186	0.022	0.016
	Power (β)	0.411	0.085	0.076
EPOC Magnitude (Kcal)	*P*	0.608	**0.004***	0.980
	η_p^2	0.018	**0.429**	0.000
	Power (β)	0.078	**0.880**	0.050
EPOC Duration (Min)	*P*	0.881	**0.031***	0.229
	η_p^2	0.096	**0.274**	0.095
	Power (β)	0.219	**0.605**	0.217
Relative Peak VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	*P*	0.226	0.166	0.166
	η_p^2	0.096	0.124	0.124
	Power (β)	0.219	0.275	0.275

η_p^2: partial eta squared; β: power; L: liters; min: minute; kcal: kilocalories; VO_2: oxygen consumption; mL: milliliters; kg: kilogram; *indicates $p<0.05$

Comparison of Pre- and Post-Training Responses

Following a two-week training intervention, there were no significant changes over time in body mass (p=0.208), body fat % (p=0.863), BMI (p=0.069), blood pressure (SBP p=0.433, DBP (p=0.957), resting HR (p=0.467), and thigh girth (Proximal Thigh p=0.846, Mid-Thigh p=0.057, Distal Thigh p=0.442). However, there were significant Differences between groups in SBP (p=0.007) as well as between groups over time in SBP (p=0.028) and Mid-Thigh Girth (p=0.015). For SBP, the Untrained individuals had a lower SBP compared to Trained individuals (p=0.028), and the Untrained individuals had a significant decrease in SBP with training while the Trained Individuals did not (p=0.007). Trained individuals had larger Mid-Thigh girths than Untrained individuals (60.56 vs. 53.69, mean difference 6.87, p=0.015). For the 3RM back squat, there were significant main effects between groups (p=0.005) and over time with training (p<0.001), as well as a significant interaction effect between groups over time (p=0.020). Overall, there was a 19.9 lb increase in 3RM squat (243.5 lbs to 260.4 lbs). Trained individuals had a higher 3RM than Untrained individuals (305.83 lbs vs 198.13 lbs, mean difference 107.7 lbs), but had a smaller increase with training (Trained 300.8 lbs to 310.8 lbs, Untrained 186.3 lbs to 210.0 lbs). Mean values and standard deviations for anthropometric measures both overall and for each group before and after training can be seen in Table 14.

Table 14. Anthropometric Data Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Overall	Trained	Untrained
Body Mass (kg)	Entry	77.2±10.7	78.5±8.9	73.0±12.1
	Exit	76.7±11.4	81.6±8.6	73.0±12.2
BMI	Entry	24.8±3.3	25.2±1.8	23.4±4.2
	Exit	24.7±3.6	26.3±1.6	23.5±4.2
BF %	Entry	13.05±4.17%	14.11±4.76%	12.46±4.05%
	Exit	12.15±4.19%	11.87±3.63%	12.36±4.80%
SBP (mmHg)	Entry	122.5±8.5	123.4±10.7	121.8±3.6
	Exit	119.2±7.8	125.5±5.0	114.5±5.93
DBP (mmHg)	Entry	73.5±6.8	73.5±9.6	72.6±5.1
	Exit	71.4±6.7	73.5±9.3	69.88±7.94
Resting HR (BPM)	Entry	69.3±12.2	70.5±10.9	69.0±15.3
	Exit	68.4±12.3	63.7±11.8	71.88±12.11
Distal Thigh Girth (cm)	Entry	38.90±2.49	39.65±2.33	37.93±2.82
	Exit	39.18±2.84	39.94±2.04	38.60±3.34
	Δ	0.34±1.35	-0.10±1.79	0.67±0.89
Mid-Thigh Girth (cm)	Entry	56.53±5.09	58.19±4.39	53.52±5.27
	Exit	56.94±5.54	61.04±4.03	53.86±4.50
	Δ	0.61±1.15	0.96±1.27	0.35±1.07
Proximal Thigh Girth (cm)	Entry	59.49±4.70	61.26±4.16	56.96±5.14
	Exit	59.10±5.46	62.11±3.89	56.84±5.57
	Δ	-0.10±1.79	-0.08±2.22	-0.11±1.55
3RM Back Squat (lbs)	Entry	243.95±77.52	272.50±72.80	186.25±52.83
	Exit	253.21±76.17*	310.83±64.22*	210.00±53.39*
	Δ	17.86±11.55	10.00±8.17	23.75±10.94

kg: kilograms; BMI: Body Mass Index; BF %: Body Fat Percentage; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; mmHg: millimeters of mercury; HR: Heart Rate; BPM: Beats Per Minute; cm: centimeters; lbs: pounds

Plasma Values

Mean values and standard deviations for plasma total spectral area, glucose, and lactate values, both overall and for each group, before and after training can be seen in Table 15. There was a significant main effect during each training session for Plasma Total Spectral Area (p=0.015) and Plasma Lactate (p=0.015) over time during each testing session. For Plasma Total Spectral Area, the Post-2 timepoint (30 minutes post exercise) was significantly greater than the Pre-exercise time point (mean difference 236.261, p=0.004). For Plasma Lactate, the Post-1 timepoint (immediately post exercise) was significantly greater than both the Post-2 timepoint (30 minutes post

exercise, mean difference 0.435, p=0.022) and the 24-Post timepoint (24 hours after exercise, mean difference 0.445, p=0.005). No other main effects or interaction effects were present for Plasma Total Spectral Area and Plasma Lactate. There were also no significant main effects or interaction effects for plasma glucose (p>0.05). Alpha levels, effect sizes, and statistical powers for comparisons in Plasma Values before and after training with low-intensity squats with BFR are shown in Table 16.

Table 15. Comparison of Plasma Values Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Pre-Training				Post-Training			
		Pre	Post-1	Post-2	24 Post	Pre	Post-1	Post-2	24 Post
Total Spectral Area	Overall	926.27 ±423.32	884.61 ±383.01	930.83 ±259.29	1028.44 ±489.47	1239.336 ±378.448	1122.741 ±211.610	889.399 ±337.321	944.849 ±321.147
	Trained	910.94 ±454.81	769.37 ±356.31	900.03 ±222.98	1088.04 ±576.70	1170.132 ±262.829	1219.855 ±204.512	788.003 ±321.825	827.435 ±332.595
	Untrained	945.43 ±410.47	1028.66 ±387.75	965.49 ±307.01	953.93 ±377.89	1291.24 ±457.67	1049.91 ±197.84	976.31 ±349.53	1032.91 ±303.06
Glucose	Overall	64.68 ±37.50	64.60 ±48.56	61.71 ±31.19	68.95 ±44.77	82.844 ±26.224	81.096 ±29.299	61.836 ±25.231	66.785 ±29.560
	Trained	60.61 ±42.99	47.23 ±42.95	58.51 ±37.88	63.66 ±52.55	80.937 ±28.245	95.899 ±26.768	50.752 ±22.790	59.541 ±29.900
	Untrained	69.76 ±31.40	86.32 ±48.85	65.30 ±23.55	75.56 ±35.02	84.27 ±26.49	69.99 ±27.46	71.34 ±24.74	72.22 ±30.09
Lactate	Overall	1.22 ±0.81	1.47 ±0.84	1.38 ±0.62	1.29 ±0.58	1.637 ±0.561	2.016 ±0.652	1.257 ±0.516	1.256 ±0.546
	Trained	1.25 ±0.95	1.30 ±0.91	1.38 ±0.72	1.33 ±0.89	1.527 ±0.408	2.262 ±0.329	1.112 ±0.485	1.099 ±0.526
	Untrained	1.16 ±0.64	1.68 ±0.75	1.38 ±0.52	1.31 ±0.75	1.72 ±0.67	1.83 ±0.79	1.38 ±0.55	1.37 ±0.56

BFR: Blood Flow Restriction

Table 16. Main Effects, Interaction Effects, Effect Size, and Power for Plasma Values Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
Total Spectral Area	*p*	0.101	**0.015***	0.364	0.122	0.873	0.887	0.296
	η_p^2	0.246	**0.291**	0.083	0.173	0.003	0.021	0.114
	Power (β)	0.372	**0.796**	0.138	0.482	0.053	0.085	0.309
Glucose	*p*	0.994	0.171	0.478	0.254	0.762	0.774	0.087
	η_p^2	0.000	0.152	0.051	0.125	0.010	0.036	0.194
	Power (β)	0.050	0.417	0.103	0.339	0.059	0.114	0.543
Lactate	*p*	0.433	**0.015***	0.872	0.130	0.404	0.915	0.239
	η_p^2	0.063	**0.294**	0.003	0.169	0.070	0.017	0.194
	Power (β)	0.115	**0.801**	0.053	0.470	0.124	0.078	0.543

BFR: Blood Flow Restriction; η_p^2: partial eta squared; β: power; *indicates $p<0.05$

Fatty Acid Moieties in the Phospholipid Bilayer

Mean values and standard deviations for fatty acid moieties in the phospholipid bilayer, both overall and for each group, before and after training can be seen in Table 17. There were no significant main effects for BFR training, over time, or between groups for any of the fatty acid moieties. There were no significant interaction effects for any variables with the exception of the V=(CH) : Vas(CH_3) Ratio, which had a significant interaction between the BFR training and training status of the individual (p=0.023). There was an increase in the ratio in untrained individuals with training (0.038 to 0.041), while the ratio decreased with training in trained individuals (0.041 to 0.038). Alpha levels, effect sizes, and statistical powers for comparisons in fatty acid moieties in the phospholipid bilayer before and after training with low-intensity squats with BFR are shown in Table 18.

Table 17. Comparison of Fatty Acid Moieties in the Phospholipid Bilayer Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Pre-Training				Post-Training			
		Pre	Post-1	Post-2	24 Post	Pre	Post-1	Post-2	24 Post
V=(CH)	Overall	0.079 ±0.014	0.092 ±0.063	0.119 ±0.115	0.118 ±0.125	0.089 ±0.027	0.076 ±0.028	0.071 ±0.034	0.072 ±0.050
	Trained	0.076 ±0.029	0.083 ±0.048	0.135 ±0.143	0.134 ±0.147	0.094 ±0.036	0.085 ±0.022	0.063 ±0.031	0.083 ±0.070
	Untrained	0.079 ±0.011	0.105 ±0.079	0.102 ±0.079	0.098 ±0.096	0.086 ±0.021	0.070 ±0.032	0.077 ±0.038	0.063 ±0.025
Vas(CH_3)	Overall	2.082 ±0.331	2.315 ±1.614	3.021 ±2.889	3.165 ±3.388	2.230 ±0.611	2.096 ±0.954	1.808 ±0.788	1.924 ±1.546
	Trained	2.061 ±0.394	2.088 ±1.353	3.332 ±3.643	3.657 ±4.023	2.393 ±0.721	2.509 ±1.024	1.625 ±0.706	2.334 ±2.229
	Untrained	2.108 ±0.255	2.597 ±1.952	2.670 ±1.908	2.551 ±2.506	2.108 ±0.531	1.786 ±0.828	1.964 ±0.875	1.572 ±0.572
Vas(CH_2)	Overall	9.982 ±1.531	11.437 ±8.116	14.338 ±13.849	14.984 ±16.057	10.665 ±2.995	10.004 ±4.499	8.538 ±3.788	8.744 ±5.088
	Trained	9.825 ±1.889	10.049 ±6.311	15.903 ±17.563	17.340 ±19.037	11.437 ±3.677	11.967 ±4.708	7.746 ±3.569	9.452 ±7.082
	Untrained	10.179 ±1.011	13.173 ±10.127	12.577 ±8.896	12.039 ±11.933	10.087 ±2.471	8.532 ±3.998	9.217 ±4.112	8.137 ±3.010
Vas(CH_2) : Vas(CH_3) Ratio	Overall	4.803 ±0.224	5.371 ±3.604	6439 ±6.614	6.605 ±6.884	4.785 ±0.168	4.784 ±0.121	4.719 ±0.112	4.893 ±1.080
	Trained	4.769 ±0.126	4.468 ±2.503	7.062 ±8.695	6.605 ±6.884	4.764 ±0.108	4.802 ±0.160	4.734 ±0.119	4.513 ±0.688
	Untrained	4.845 ±0.312	6.500 ±4.569	5.739 ±3.537	7.192 ±8.529	4.800 ±0.209	0.165 ±0.051	4.707 ±0.113	5.218 ±1.293
V=(CH) : Vas(CH_3) Ratio	Overall	0.038 ±0.002	0.041 ±0.005	0.040 ±0.006	0.038 ±0.005	0.040 ±0.004	0.038 ±0.008	0.038 ±0.002	0.038 ±0.007
	Trained	0.038 ±0.002	0.041 ±0.005	0.042 ±0.006	0.037 ±0.005	0.039 ±0.003	0.037 ±0.012	0.038 ±0.002	0.036 ±0.007
	Untrained	0.037 ±0.002	0.040 ±0.006	0.037 ±0.004	0.038 ±0.004	0.041 ±0.003	0.039 ±0.003	0.039 ±0.003	0.041 ±0.007
V(P=O)	Overall	1.555 ±0.280	1.736 ±1.054	2.285 ±2.494	2.251 ±2.403	1.675 ±0.482	1.524 ±0.677	1.329 ±0.642	1.451 ±0.907
	Trained	1.557 ±0.337	1.598 ±0.886	2.689 ±3.263	2.544 ±2.831	1.767 ±0.631	1.824 ±0.706	1.196 ±0.602	1.562 ±1.231
	Untrained	1.553 ±0.209	1.908 ±1.276	1.831 ±1.269	1.885 ±1.854	1.605 ±0.367	1.300 ±0.601	1.443 ±0.699	1.356 ±0.602
RBC Glucose	Overall	0.196 ±0.048	0.227 ±0.151	0.299 ±0.344	0.295 ±0.316	0.212 ±0.062	0.190 ±0.084	0.166 ±0.083	0.179 ±0.115
	Trained	0.203 ±0.057	0.211 ±0.116	0.358 ±0.456	0.336 ±0.370	0.218 ±0.078	0.226 ±0.085	0.150 ±0.075	0.198 ±0.160
	Untrained	0.187 ±0.034	0.246 ±0.192	0.232 ±0.152	0.244 ±0.248	0.208 ±0.052	0.162 ±0.077	0.181 ±0.092	0.162 ±0.067
RBC Lactate	Overall	0.236 ±0.044	0.288 ±0.228	0.350 ±0.360	0.369 ±0.368	0.250 ±0.075	0.230 ±0.097	0.196 ±0.096	0.219 ±0.134
	Trained	0.240 ±0.055	0.255 ±0.150	0.404 ±0.469	0.422 ±0.411	0.263 ±0.091	0.283 ±0.089	0.182 ±0.087	0.234 ±0.178
	Untrained	0.231 ±0.026	0.329 ±0.307	0.290 ±0.190	0.302 ±0.320	0.241 ±0.065	0.190 ±0.088	0.209 ±0.109	0.207 ±0.096

v: stretching vibrations; *s*: symmetric; *as*: asymmetric; RBC: Red Blood Cell

Table 18. Main Effects, Interaction Effects, Effect Size, and Power for Fatty Acid Moieties in the Phospholipid Bilayer Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
V=(CH)	*p*	0.573	0.771	0.331	0.393	0.533	0.292	0.284
	η_p^2	0.037	0.018	0.105	0.091	0.045	0.126	0.130
	Power (β)	0.082	0.068	0.151	0.155	0.090	0.202	0.208
Vas(CH_3)	*p*	0.621	0.682	0.296	0.356	0.702	0.275	0.300
	η_p^2	0.028	0.029	0.120	0.103	0.017	0.133	0.123
	Power (β)	0.074	0.079	0.169	0.168	0.065	0.210	0.196
Vas(CH_2)	*p*	0.551	0.682	0.353	0.302	0.614	0.355	0.206
	η_p^2	0.041	0.029	0.096	0.122	0.029	0.100	0.168
	Power (β)	0.086	0.079	0.142	0.191	0.076	0.157	0.259
Vas(CH_2) : Vas(CH_3) Ratio	*p*	0.522	0.587	0.478	0.550	0.079	0.461	0.146
	η_p^2	0.047	0.039	0.057	0.047	0.304	0.067	0.214
	Power (β)	0.092	0.087	0.102	0.095	0.426	0.116	0.312
V=(CH) : Vas(CH_3) Ratio	*p*	0.819	0.528	0.622	0.430	**0.023***	0.650	0.580
	η_p^2	0.006	0.069	0.028	0.096	**0.453**	0.047	0.069
	Power (β)	0.055	0.145	0.074	0.231	**0.681**	0.112	0.171
V(P=O)	*p*	0.562	0.737	0.324	0.399	0.548	0.414	0.226
	η_p^2	0.039	0.018	0.108	0.089	0.042	0.081	0.157
	Power (β)	0.084	0.067	0.155	0.149	0.087	0.134	0.245
RBC Glucose	*p*	0.501	0.684	0.285	0.362	0.485	0.398	0.262
	η_p^2	0.052	0.024	0.125	0.100	0.056	0.086	0.139
	Power (β)	0.096	0.073	0.175	0.162	0.100	0.138	0.215
RBC Lactate	*p*	0.516	0.690	0.306	0.350	0.560	0.429	0.224
	η_p^2	0.048	0.024	0.116	0.105	0.039	0.077	0.157
	Power (β)	0.093	0.073	0.164	0.174	0.084	0.130	0.251

v: stretching vibrations; *s*: symmetric; *as*: asymmetric; η_p^2: partial eta squared; β: power; RBC: Red Blood Cell; * indicates significance at the *p*=0.05 level

Proteins in Erythrocytes

Mean values and standard deviations for proteins in erythrocytes, both overall and for each group, before and after training can be seen in Table 19. There was a significant Time main effect for V(C=O), with the Post-2 (30 minutes post-exercise) time point being significantly smaller than the 24 Post (24 hours post exercise) time point (mean difference -0.129, *p*=0.049). Alpha levels, effect sizes, and statistical powers for comparisons in erythrocyte proteins before and after training with low-intensity squats with BFR are shown in Table 20.

Table 19. Comparison of Proteins in Erythrocytes Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Pre-Training				Post-Training			
		Pre	Post-1	Post-2	24 Post	Pre	Post-1	Post-2	24 Post
V(C=O)	Overall	0.200 ±0.229	0.230 ±0.418	0.167 ±0.191	0.226 ±0.209	0.173 ±0.111	0.132 ±0.063	0.091 ±0.062	0.238 ±0.309
	Trained	0.142 ±0.106	0.076 ±0.066	0.194 ±0.189	0.282 ±0.247	0.123 ±0.136	0.118 ±0.080	0.074 ±0.085	0.184 ±0.154
	Untrained	0.272 ±0.319	0.421 ±0.585	0.136 ±0.202	0.155 ±0.130	0.210 ±0.077	0.143 ±0.050	0.106 ±0.034	0.284 ±0.407
Amide I V(C=O)	Overall	69.777 ±14.864	78.035 ±55.568	98.040 ±74.121	112.392 ±118.336	74.961 ±18.271	78.071 ±37.762	60.859 ±21.716	57.657 ±35.042
	Trained	69.332 ±15.737	70.392 ±54.506	102.354 ±88.512	134.193 ±144.270	84.899 ±13.927	94.770 ±42.509	55.501 ±18.938	65.154 ±45.977
	Untrained	70.333 ±14.749	87.590 ±59.089	93.186 ±59.607	85.140 ±75.631	67.508 ±18.257	65.547 ±30.647	65.452 ±24.310	51.232 ±24.287
Amide II (ō-N)	Overall	38.259 ±6.439	42.420 ±30.280	53.823 ±43.528	60.474 ±65.763	40.910 ±10.475	40.955 ±19.539	32.985 ±12.055	31.284 ±19.871
	Trained	37.752 ±7.135	37.621 ±27.396	56.685 ±51.963	71.367 ±79.790	45.252 ±10.475	49.435 ±21.541	29.978 ±10.602	36.249 ±26.182
	Untrained	38.892 ±5.866	48.418 ±34.462	50.603 ±34.965	46.857 ±44.003	37.653 ±9.594	34.595 ±16.422	35.563 ±13.423	27.028 ±13.142
Amide I : Amide II Ratio	Overall	1.812 ±0.142	1.824 ±0.142	1.850 ±0.142	1.871 ±0.138	1.839 ±0.152	1.901 ±0.136	1.860 ±0.138	1.889 ±0.136
	Trained	1.826 ±0.161	1.815 ±0.147	1.827 ±0.150	1.884 ±0.139	1.901 ±0.140	1.889 ±1.54	1.860 ±0.123	1.839 ±0.095
	Untrained	1.794 ±0.121	1.836 ±0.144	1.875 ±0.138	1.854 ±0.144	1.792 ±0.152	1.909 ±0.130	1.860 ±0.160	1.931 ±0.157

v: stretching vibrations; *δ*: bending (scissoring) vibrations

Table 20. Main Effects, Interaction Effects, Effect Size, and Power for Erythrocyte Proteins Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
V(C=O)	*p*	0.821	**0.038***	0.576	0.772	0.213	0.291	0.395
	η_p^2	0.006	**0.264**	0.036	0.015	0.166	0.127	0.087
	Power (β)	0.055	**0.677**	0.082	0.064	0.225	0.310	0.142
Amide I V(C=O)	*p*	0.627	0.801	0.384	0.152	0.889	0.298	0.210
	η_p^2	0.027	0.013	0.085	0.208	0.002	0.123	0.167
	Power (β)	0.074	0.062	0.130	0.306	0.052	0.192	0.245
Amide II (ō-N)	*p*	0.582	0.796	0.359	0.188	0.798	0.280	0.232
	η_p^2	0.035	0.012	0.094	0.181	0.008	0.130	0.154
	Power (β)	0.081	0.062	0.140	0.268	0.056	0.199	0.230
Amide I : Amide II Ratio	*p*	0.125	0.467	0.324	0.421	0.155	0.611	0.729
	η_p^2	0.241	0.088	0.108	0.097	0.211	0.064	0.046
	Power (β)	0.3258	0.215	0.155	0.235	0.284	0.161	0.126

v: stretching vibrations; *δ*: bending (scissoring) vibrations; η_p^2: partial eta squared; β: power; *indicates $p<0.05$

Cortisol Salivary Markers

Mean values and standard deviations for markers of salivary cortisol both overall and for each group can be seen in Table 21. There were no significant main effects before and after training, over time during each testing session, or between training statuses as well as no significant interaction effects between these main effects (p>0.05) with the exception of Session by Time interaction for the 3500-3200 cm^{-1} band (p=0.032). Prior to training, the Post-2 timepoint was significantly higher than the Post-1 timepoint, but this change was not significant following training. Alpha levels, effect sizes, and statistical powers for comparisons in cortisol salivary markers between low-intensity squats with BFR and traditional high-intensity squats are shown in Table 22.

Table 21. Comparison of Markers for Salivary Cortisol Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Pre-Training			Post-Training		
		Pre	Post-1	Post-2	Pre	Post-1	Post-2
3500-3200 cm^{-1}	Overall	47.220 ±26.913	38.813 ±20.950	57.036 ±27.989	44.598 ±28.215	44.267 ±29.271	27.960 ±14.992
	Trained	48.374 ±22.449	37.418 ±22.487	50.565 ±28.874	63.339 ±31.532	45.698 ±25.342	26.268 ±8.609
	Untrained	45.778 ±33.269	40.556 ±20.236	63.507 ±27.373	32.885 ±19.721	43.193 ±33.607	29.410 ±19.555
1900-900 cm^{-1}	Overall	21.910 ±8.333	21.600 ±10.135	26.129 ±8.381	20.178 ±10.333	21.461 ±10.934	17.683 ±11.299
	Trained	21.627 ±6.388	19.653 ±9.404	24.899 ±5.525	20.988 ±7.781	20.780 ±7.549	15.625 ±9.200
	Untrained	22.263 ±10.766	24.034 ±11.117	27.360 ±10.794	19.672 ±12.152	21.972 ±13.440	19.447 ±13.302
1946-1526 cm^{-1}	Overall	10.855 ±4.144	10.637 ±4.500	13.028 ±4.160	9.908 ±5.141	10.721 ±5.698	8.414 ±5.245
	Trained	10.856 ±2.974	9.923 ±4.353	11.993 ±2.933	11.092 ±4.128	10.812 ±4.357	7.639 ±4.156
	Untrained	10.852 ±5.508	11.529 ±4.814	14.062 ±5.102	9.167 ±5.826	10.653 ±6.835	9.078 ±6.286
1391-1249 cm^{-1}	Overall	2.612 ±4.085	24.09 ±1.399	3.114 ±1.051	2.381 ±1.468	2.420 ±1.341	2.139 ±1.684
	Trained	2.489 ±0.794	2.162 ±1.314	3.080 ±0.655	2.340 ±1.011	2.225 ±0.885	1.683 ±1.255
	Untrained	2.766 ±1.415	2.717 ±1.530	3.149 ±1.392	2.406 ±1.763	2.565 ±1.650	2.529 ±1.994
1115-973 cm^{-1}	Overall	2.950 ±1.016	3.008 ±1.499	3.442 ±1.096	2.814 ±1.428	2.903 ±1.379	2.596 ±1.648
	Trained	2.938 ±0.898	2.613 ±1.286	3.400 ±0.611	2.665 ±0.864	2.738 ±0.912	2.389 ±1.479
	Untrained	2.966 ±1.211	3.501 ±1.683	3.483 ±1.482	2.907 ±1.736	29.410 ±19.555	2.773 ±1.879

Table 22. Main Effects, Interaction Effects, Effect Size, and Power for Salivary Cortisol Markers Before and after a Two-Week Intervention of Low-Intensity Squats with BFR

		Session	Time	Training Status	Session * Time	Session * Training Status	Time * Training Status	Session * Time * Training Status
3500-3200 cm^{-1}	*p*	0.208	0.616	0.528	**0.032***	0.929	0.460	0.146
	η_p^2	0.170	0.052	0.046	**0.318**	0.001	0.083	0.193
	Power (β)	0.229	0.119	0.091	**0.661**	0.051	0.167	0.382
1900-900 cm^{-1}	*p*	0.240	0.616	0.951	0.239	0.496	0.890	0.752
	η_p^2	0.149	0.052	0.000	0.147	0.053	0.013	0.031
	Power (β)	0.203	0.119	0.050	0.286	0.098	0.065	0.089
1946-1526 cm^{-1}	*p*	0.201	0.861	0.890	0.184	0.729	0.929	0.643
	η_p^2	0.174	0.017	0.002	0.172	0.014	0.008	0.048
	Power (β)	0.235	0.070	0.052	0.336	0.062	0.060	0.112
1391-1249 cm^{-1}	*p*	0.343	0.407	0.951	0.285	0.347	0.856	0.652
	η_p^2	0.100	0.095	0.000	0.130	0.099	0.017	0.046
	Power (β)	0.146	0.188	0.050	0.253	0.144	0.072	0.110
1115-973 cm^{-1}	*p*	0.333	0.350	0.762	0.289	0.995	0.189	0.912
	η_p^2	0.094	0.100	0.010	0.117	0.000	0.153	0.009
	Power (β)	0.152	0.217	0.059	0.252	0.050	0.333	0.062

η_p^2: partial eta squared; β: power; * indicates significance at the *p*=0.05 level

Excessive Post-Exercise Oxygen Consumption

Mean values and standard deviations for markers of salivary cortisol both overall and for each group can be seen in Table 23. There were no significant differences in resting oxygen consumption or peak oxygen consumption during exercise before and after training and between Trained vs Untrained individuals (*p*>0.05 for main and interaction effects, see Table 24). While there were no significant main effects between conditions, nor in Session by Group interactions for either measure of EPOC (*p*>0.05 for all), the Trained individuals had a significantly higher magnitude of EPOC (23.134 kcal vs 14.364 kcal, mean difference 8.770 kcals, *p*=0.005) and duration of EPOC (18.55 min vs. 13.45 min, mean difference 5.10 min, *p*=0.030).

Table 23. Comparison of Excessive Post-Exercise Oxygen Consumption Following Traditional High-Intensity Back Squats and Low-Intensity Back Squats with BFR Before and After a Two-Week Training Intervention

		Pre-Training	Post-Training
Resting VO_2 ($L \cdot min^{-1}$)	Overall	0.330±0.143	0.292±0.047
	Trained	0.391±0.210	0.318±0.056
	Untrained	0.308±0.094	0.280±0.038
Magnitude (Kcal)	Overall	16.73±7.29	17.549±7.611
	Trained	17.78±4.74	21.215±3.105
	Untrained	12.76±5.89	14.560±8.822
Duration (Min)	Overall	14.16±5.78	16.560±5.421
	Trained	14.45±6.86	17.153±4.045
	Untrained	13.37±6.11	15.639±6.422
Relative Peak VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	Overall	29.95±4.33	28.218±4.015
	Trained	33.30±3.22	30.040±3.984
	Untrained	28.56±4.43	26.897±4.026

L: liters; min: minute; kcal: kilocalories; VO_2: oxygen consumption; mL: milliliters; kg: kilogram

Table 24. Main Effects, Interaction Effects, Effect Size, and Power for Oxygen Consumption Before, During, and After Low-Intensity Squats with BFR and Traditional High-Intensity Squats

		Session	Training Status	Session * Training Status
Resting VO_2 ($L \cdot min^{-1}$)	*p*	0.552	0.296	0.917
	η_p^2	0.030	0.090	0.001
	Power (β)	0.087	0.172	0.051
EPOC Magnitude (Kcal)	*p*	0.563	**0.005***	0.461
	η_p^2	0.029	**0.493**	0.046
	Power (β)	0.085	**0.880**	0.108
EPOC Duration (Min)	*p*	0.487	**0.030***	0.165
	η_p^2	0.041	**0.337**	0.154
	Power (β)	0.101	**0.621**	0.275
Relative Peak VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	*p*	0.486	0.265	0.380
	η_p^2	0.041	0.102	0.065
	Power (β)	0.102	0.190	0.134

η_p^2: partial eta squared; β: power; L: liters; min: minute; kcal: kilocalories; VO_2: oxygen consumption; mL: milliliters; kg: kilogram; *indicates $p<0.05$

Discussion

Low-intensity Squats with BFR versus Traditional High-intensity Squats

Oxidative Stress

The most important finding in the comparison between the two different exercise modalities in the present study is the lack of any significant changes in fatty acid moieties or proteins in erythrocytes. Petibois et al. had previously reported alterations to both the phospholipid bilayer and proteins in erythrocytes during sustained aerobic exercise (two hours of rowing at 70% of VO_{2MAX}). It is most likely that this effect wasn't seen in the present study due to the fact that the exercises utilized did not expose erythrocytes to sufficient oxygen flux to cause a significant increase in ROS exposure. The V(C=O) bond that had significant Time by Training Status and Condition by Time by Training Status interactions is indicative of phospholipids and cholesterol esters in erythrocytes. This bond was not one of those reported by Petibois et al. as one of those undergoing significant changes with aerobic exercise [48]. It is possible that these changes in the V(C=O) bond could be due to movement of phosphatidylinositol 4,5-bisphosphate (PIP2) and Phosphatidylinositol (3,4,5)-triphosphate (PIP3), a phospholipid in the plasma membrane involved in anabolic cell signaling.

This lack of changes in oxidative stress with BFR exercise supports other findings in the literature. Neto et al. reported no differences in blood markers of oxidative stress responses with low-intensity resistance exercise with BFR [89], as well as Behringer et al. (2017) who reported no changes in metabolic stress, endocrine response nor indirect markers of muscle damage with eccentric resistance training with BFR [90]. Yanagisawa and Sanomura (2017) reported decreased intracellular pH,

tissue oxygenation and phosphocreatine levels with resistance training with BFR [91]. Layne et al. (2017) reported that exercise with BFR increased myogenic gene expression but decreased Human Growth Factor protein expression up to 24 hours post exercise compared to controls [92]. A recent review by Dankel et al (2017) concluded that the increased metabolites from BFR training supplement the mechanical pathways for muscle growth by increasing the number of muscle fibers activated and speeding the onset of muscular fatigue [93]. This increase in fatigue is potentially caused by the greater time under tension of a greater number of muscle fibers, which allows for activation of the calcium-calmodulin protein kinase II (CaMKII) pathway, which in turn is thought to play a role upstream of the mTORC1 pathway. Dankel et al concluded that the metabolites pooled during resistance training during BFR may not have direct anabolic properties, but instead enhance activation of muscle fibers, thus increasing hypertrophy through the same mTORC1 pathway utilized during high-intensity resistance training [93].

Markers of Salivary Cortisol

The significant increase seen in the 3500-3200 cm^{-1} spectral band following low-intensity exercise with BFR could potentially be explained by the fact that while the external load was volume matched between conditions, the low-intensity exercise consisted of a considerably greater number of actual back squats (75 repetitions compared to 32 repetitions). Additionally, the changes in the 1900-900 cm^{-1} and 1946-1526 cm^{-1} approached significance for Condition by Time interaction (p=0.098 and 0.058 respectively), with both markers again rising at Post-2 (30 minutes post exercise) time point with low-intensity BFR exercise, but not the traditional high-intensity exercise.

Thus, it appears that low-intensity exercise with BFR causes an increase in salivary cortisol at 30 minutes following exercise. This is in agreement with Kraemer and Ratamess (2005), who reported that high volume, moderate to high intensity exercises stressing a large muscle mass elicit an increase in cortisol levels 15 to 30 minutes post-exercise [94].

Excessive Post-Exercise Oxygen Consumption

Trained individuals underwent a significantly longer EPOC compared to Untrained individuals regardless of the exercise intervention (20.90 min vs. 13.46 min). This most likely also contributed to the significant difference in the magnitude of EPOC between Trained and Untrained individuals as well (16.43 kcals vs. 12.32 kcals). This difference between groups could be due to the increased load during exercise (Low intensity: 89.00±24.01 vs. 61.25±16.42 lbs, High Intensity: 207.50±55.87 vs. 141.88±38.91 lbs) as well as differences in the muscle cross sectional area that provided this difference in force production.

Effects of Two Weeks of Training with Low-intensity Squats with BFR

Anthropometrics and Strength

While other studies have reported increases in thigh girth with low-intensity resistance training with BFR [11, 95], these results were not seen in the present study. This could potentially be due to the short-term nature of the training (two weeks) as opposed to other studies which were normally conducted over four weeks or more; however, another study by Luebbers et al. utilizing supplemental training with BFR reported no changes in girth measurements over seven weeks of training [9]. The

strength gains reported in the present study agrees with the results of Sousa et al. (2017), who reported that while torque from high-intensity resistance training didn't increase until after six weeks of training, low-intensity exercise with BFR had significant increases in lower extremity torque after only two weeks [96].

Oxidative Stress

Plasma total spectra area significantly decreased at the Post-2 (30 minutes post exercise) time point, most likely due to a plasma volume shift into the muscles. However, this shift normally peaks immediately post exercise and gradually returns to normal over the course of an hour [97]. The significant Session-by-Training-Status interaction effect for the V=(CH) : Vas(CH_3) Ratio, which represents damage to the phospholipid bilayer in erythrocytes, indicated a significant decrease in the Trained, but not Untrained individuals with two weeks of low-intensity back squats with BFR training. Unlike the comparison between the low-intensity with BFR and traditional high-intensity exercises, where there was no Time-by-Training-Status and Session-by-Time-by-Training-Status interaction effects, but there was a significant main effect for time, with a significant increase in the V(C=O) bond, a marker of phospholipids and cholesterol esters in the erythrocyte. These changes were not nearly as extensive as the changes reported with aerobic exercise training by Petibois et al. [49, 55].

Markers of Salivary Cortisol

As with comparisons between low-intensity back squats with BFR and traditional high-intensity back squats, the significant Session-by-Time interaction was still present when comparing the results of testing before and after two weeks of BFR training. The

significant increase in the 3500-3200 cm^{-1} spectral band at the Post-2 timepoint was present prior to training, but not present following two weeks of training. Thus, in can be concluded that two weeks of training with low-intensity back squats with BFR can reduce the salivary cortisol response to this exercise modality. Kraemer and Ratamess also described an up-regulation in glucocorticoid receptors and a corresponding decrease in the magnitude of cortisol response to exercise following subsequent training bouts [94].

Excessive Post Exercise Oxygen Consumption

Once again, there were significant differences between the Trained and Untrained individuals for EPOC duration and magnitude. The duration of EPOC was significantly longer in Trained individuals (18.55 min vs. 13.45 min), as well as the magnitude (23.13 kcal vs. 14.36 kcal). Since there were no significant differences in body mass or body fat percentage between the two groups, it could be that the differences are derived from the heavier load used by the Trained individuals (89.00±24.01 vs. 61.25±16.42 lbs), which would amount to a mean increase in the volume of training over the course of an exercise session of 2,081 lbs.

Limitations

One of the primary limitations of the study was the lack of control for an individual's lifestyle over the course of the study. While participants were asked to adhere to some dietary and exercise restrictions during the study, their adherence cannot be confirmed. Due to the small effect sizes reported, this study was underpowered, and the authors are unable to verify the absence of Type II errors for most nonsignificant findings. Additionally, the use of volumetric measuring for the

dilution of plasma and erythrocyte samples prior to desiccation could be a potential source of error.

Conclusion

There were fewer changes in measures of oxidative stress in erythrocytes with resistance training (both low-intensity back squats with BFR and high-intensity back squats) than with high-intensity aerobic exercise. This is potentially due to the lower amount of oxygen flux endured by erythrocytes during high-intensity aerobic exercise. Additionally, recent studies have concluded that the main metabolic markers for hypertrophy during resistance training appear to come from mechanical activation of the CaMKII and mTORC1 pathways and fatigue, rather than other metabolic pathways [93]. Markers of salivary cortisol (specifically the 3500-3200 cm^{-1} spectral band) were significantly increased following low intensity exercise with BFR before, but not after two weeks of resistance training. Cortisol markers did not change significantly with traditional high-intensity back squat exercise. There were no differences between the two types of exercise and no changes due to training in EPOC magnitude and duration, however, there was a significant difference between Trained and Untrained individuals, most likely due to the volume differences between groups. Finally, two weeks of training with low-intensity back squats with BFR significantly increased strength in both Trained and Untrained males. It was concluded that two weeks of low-intensity back squats with BFR significantly increased strength in both Trained and Untrained males without significant increases in thigh girth or erythrocyte oxidative stress.

Part II

Review of Literature

Blood Flow Restriction

Blood Flow Restriction training has become a growing topic of interest in recent years and has received more exposure and use in both the strength and conditioning and the rehabilitation fields. It has been used in several different populations to either supplement and/or replace traditional strength training. While it has shown promising results, the mechanisms behind some of these adaptations to training remains unclear.

Acute Blood Flow Restriction

In response to acute training with Blood Flow Restriction, several potential mechanisms have been described, such as metabolic accumulation, increased activation of type II muscle fibers, increased oxidative stress, etc. [12, 13, 86]. Several studies have also examined the safety of Blood Flow Restriction, since occlusive training does have an increased potential for adverse side effects [75, 76, 98]. Additionally, a standardized training protocol for Blood Flow Restriction training has yet to be developed.

Grassi et al. investigated the effects that incremental exercise on a cycle ergometer had on muscle oxygenation and blood lactate accumulation in five well-trained mountain climbers [99]. Metabolic measurements (ventilation, VO_2, VCO_2) were taken at rest and during the last 30-45 seconds of each stage, as well as arterial blood saturation, heart rate, and vastus lateralis oxygenation. A significant correlation was found between the point of increase for blood lactate and the point where muscle

oxygenation begins to decrease. The authors concluded that the muscle deoxygenation observed at workloads >60-65% of VO_{2MAX} is most likely due to an accelerate capillary-venous hemoglobin desaturation which led to a rightward shift of oxygen hemoglobin binding curve due to the build-up of lactic acid [99].

McCaulley et al. examined the neuroendocrine responses to hypertrophy, strength, and power type resistance exercises in 10 male subjects controlled for volume. Participants completed four lower-body resistance training protocols in a random order 24 to 48 hours apart. The one repetition maximum (1RM) for back squat was tested prior to the start of the study. The hypertrophy protocol consisted of four sets of 10 repetitions (reps) at 70% of 1RM with 90 seconds (s) between sets; the strength protocol was 11 sets of 3 reps at 90% of 1RM with five minutes between sets; the power protocol consisted of eight sets of six squat jumps (resistance was body mass) with three minutes of rest between sets; while the fourth protocol was a control day during which the participant rested quietly. Volume was calculated as concentric and eccentric work. Blood samples were collected before exercise, and 0, 1, 24, and 48 hours post exercise and analyzed for lactate, testosterone, cortisol, and sex hormone binding globulin. Maximal isometric squats were also performed pre-, immediately after, and 1, 24, and 48 hours post-exercise. Muscle activity was recorded using EMG. The hypertrophy protocol had significantly increased change in all hormones recorded compared to controls ($p<0.05$). There was a dose-response relationship between metabolic demand of the protocol and change in hormone concentrations from resting to immediately post-exercise. Both the strength and hypertrophy protocols resulted in decreased force and rate of force development immediately after exercise compared to

controls (p<0.05), the power and resting protocols did not create any changes in force variables. There were no differences for all hormones at 24 or 48 hours post exercise for any training protocol. The authors concluded that the total volume of resistance exercise is not the main variable in eliciting acute hormonal response. They theorized that hypertrophy protocols may create internal muscular environment similar to BFR [5].

Cook, Murphy, and Labarbera compared endurance, torque decrement, central activation, muscle activation and evoked contractile function a single bout of low load, high load, and low load with BFR knee extensions to fatigue in eight recreationally active males [2]. Low load knee extensions were performed at 20% of peak dynamic torque and high load knee extensions were performed at 70% of peak dynamic torque. Participants performed three sets of unilateral knee extensions with a concentric/eccentric pace of 2 seconds to fatigue with 30 seconds of rest between sets. A within subjects repeated measures ANOVA with Bonferroni post-hoc was used to assess differences between conditions (time by group). Participants performed more repetitions in the low load and low load with BFR compared to high load (p=0.01, η_p^2=0.48); however, there was no significant difference in exercise volume between conditions (p=0.07, η_p^2=0.27). Peak isometric torque was similar prior to exercise with all conditions, but decreased similarly following exercise (37%, p<0.01, η_p^2=0.94), but again did not differ significantly between conditions (p=0.35, η_p^2=0.14). Central activation changed by 3.8%, -6.1%, -3.6% following exercise for high load, low load, and low load with BFR respectively (p=0.1, η_p^2=0.34). The high load condition consistently had increased muscle activity than the low load (p=0.02) and low load with BFR (p=0.04) before and after exercise. There were no significant differences between

groups in heart rate or ratings of perceived of exertion (p>0.05). The authors concluded that all three conditions resulted in similar deficits in torque despite differences in muscle activation and volume and that the fatigue with exercise to peripheral factors due to the fact that central activation was unchanged [2].

Loenneke et al. (2012a) investigated the potential reasons for BFR responses without exercise in 10 (5 male, 5 female) participants [3]. Anthropometrics (height, body mass, thigh circumference, and resting blood pressure) were all measured during the initial visit. During the second visit, the BFR cuffs were placed in position but not fastened or tightened while EMG, HR, muscle thickness, blood lactate and hematocrit were measured after 10 minutes of rest and another 10 minutes of control. The BFR cuffs (KAATSU, 5 cm) were then fastened and inflated for five-minute bouts at 70% of the participants predicted occlusion pressure with 3 minutes of deflation between bouts with EMG, HR, muscle thickness being measured 1 minute prior to all inflations and deflations. Blood samples were also taken at the end of the last inflation and 5 minutes after the last inflation along with the other variables. Plasma volume decreased by 15% (p=0.001), while significant increases in muscle thickness was seen in the vastus lateralis (6%, p=0.027) and rectus femoris (22%, p=0.001). Blood lactate, electromyography, and heart rate did not change significantly. Ratings of discomfort peaked at light discomfort (2.7/10). The authors concluded that BFR, even without exercise, causes an acute fluid shift, leading to an increase in muscle mass. Additionally, it was determined that metabolic accumulation (namely blood lactate) did not occur in BFR with the absence of exercise [3].

Loenneke et al. (2013) investigated decrements in lower extremity torque following low-intensity resistance exercise with and without BFR in 16 healthy participants [4]. Exercise consisted of 4 set of knee extensions (30, 15, 15, and 15 reps) against 30% of 1RM separated by 30 seconds of rest. Participants performed maximal voluntary contractions before and immediately after exercise, as well as 1 hour and 24 hours post-exercise. Those selected for the BFR group had a 5 cm (KAATSU) wide cuff placed on the proximal thigh that was inflated to 120, 150, 180, or 210 mmHg depending on thigh circumference. Repeated measures ANOVAs were used to assess differences between groups. Low-intensity exercise produced an acute drop in MVC immediately after exercise that rebounded quickly (p=0.03). This was due to the intensifying effects of BFR on exercise, rather than BFR itself, since BFR during rest did not produce changes in MVC. The authors concluded that BFR, alone or with low-intensity exercise, can be safely applied in healthy participants without reducing muscle function [4].

Araújo et al. examined the acute effects of low-intensity resistance training with BFR and moderate-intensity resistance training on blood pressure and heart rate before, during, and after exercise in 14 untrained females with type 1 hypertension [1]. Anthropometrics and 1RM were collected prior to the start of the study. Participants performed three sets of 15 repetitions knee extensions at a low-intensity (30% of 1RM) with 80% occlusion (18 cm wide cuff) and 45 seconds between sets and a moderate-intensity (80% of 1RM) with 1 minute between each set. Blood pressure and heart rate were measured prior to, immediately after each set, and 15, 30, 45, and 60 minutes post exercise. Repeated measures ANOVAs were used to assess differences between

conditions and over time. Low-intensity exercise with BFR resulted significant decreases in SBP at every time points post exercise ($p<0.05$). During exercise, a significant decrease in DBP was observed between the first and second set with moderate intensity exercise but between the second and third set for low-intensity exercise with BFR ($p<0.05$ for both). The authors concluded that low-intensity exercise with BFR produced a hypotensive effect for up to 60 minutes post-exercise [1].

Chulvi-Medrano wrote a letter in response to Araujo et al.'s paper on the post-exercise hypotensive effects in hypertensive subjects following BFR resistance exercise to reiterate the safety of this type of training in regards to blood pressure. He brings up several areas of further research needed to further the knowledge of the physiological effects of BFR resistance exercise on hypertensive subjects: resistance training with different levels of blood flow restriction; different levels of resistance training; BFR training versus traditional resistance and aerobic training method [100].

Jessee et al. investigated the differences in arterial occlusion pressure using three common cuff widths in 249 participants in order to determine the differences caused by sex and race and establish prediction equations for the occlusion pressure using each cuff width [101]. Resting blood pressure was measured and the standing arterial occlusive pressure for each cuff width (5, 10, and 12 cm wide) was determined using Doppler ultrasound. Anthropometrics (height, body mass, upper limb length and circumference) were also collected. A repeated measures ANOVA was used to determine differences in occlusive pressures between cuffs and hierarchical linear regression was used to determine the best predictors for occlusive pressures and produce prediction equations. Significant differences were found in the occlusive

pressures for each cuff, with the occlusive pressure decreasing as the cuff increased in width (p<0.001). The magnitude of differences was not large between sexes. Non-Hispanic blacks had higher occlusive pressures than whites (p<0.001 for 5, 10, and 12 cm cuffs). Predictive equations for all three cuff widths had no significant difference between predicted and actual occlusive pressures. The final occlusive prediction equations were as follows: Occlusive pressure (5 cm) = 2.790 * (arm circumference) + 1.119 * (SBP) – 0.439 * (arm length) + 0.224 * (DBP) + 12.467 * (sex) – 77.636, occlusive pressure (10 cm) = 1.521 * (arm circumference) + 0.833 * (SBP) – 0.296 * (arm length) + 0.139 * (DBP) + 6.869 (sex) – 21.344, occlusive pressure (12 cm) = 1.444 * (arm circumference) + 0.833 * (SBP) – 0.297 * (arm length) + 0.159 * (DBP) + 7.355 * (sex) – 13.216. The researchers concluded that it is important to report cuff width in the literature because differences in cuff width can cause changes in the percentage of arterial flow restricted under different pressures and put forth the occlusive prediction equations for potential use in predicting occlusive pressure and determining pressure for BFR for future research [101].

Acute resistance training with BFR has been shown to achieve similar acute changes in muscle size, fatigue, hypotensive response, and recovery as traditional high intensity training methods [1-5]. Thus resistance training with BFR can cause similar effects in strength and neuromuscular control at a much lower intensity then traditional resistance exercise. Making low-intensity resistance exercise with BFR a viable alternative when individuals are unable to comply with a normal resistance training protocol. The occlusive pressure needed for BFR varies in relation to both the limb type and cuff size used [101].

Acute BFR with Treadmill Walking

Blood Flow Restriction has also been used with treadmill walking in order to increase the intensity of the exercise without increasing the speed or incline of the treadmill and has been shown to increase muscular strength and hypertrophy compared to controls [66, 67]. Due to the aerobic nature of the exercise, this exercise protocol has primarily been investigated using metabolic data.

Mendonca et al. investigated the effects of interval BFR walking on the net metabolic cost of walking in 18 healthy young males and attempted to determine if ventilatory equivalents for VO2 and VCO_2 was related to changes in walking economy; and if RPE dictated the magnitude of central command [68]. Participants completed three visits separated by at least 48 hours. During the first visit, anthropometrics were collected, optimal walking speed was determined, and VO_{2MAX} was measured. On the following visits, participants walked at their optimal walking speed with and without BFR while collecting data with a metabolic cart. These trials consisted of five bouts of 3 minutes of walking with 1 minute of rest in between sets. Blood flow was restricted using a 6 cm wide that was inflated to a final pressure of 200 mmHg; this pressure was maintained throughout the walking trials, including during the rest periods. Treadmill walking with BFR was found to increase the net cost of walking and increase ventilation, most likely in response to increased VCO_2. There appeared to be no relationship between RPE and ventilation [68].

Having previously determined that treadmill walking with BFR increased ventilation and the net cost of walking, Mendonca et al. then investigated the effects of treadmill walking with BFR on excessive post-exercise oxygen consumption in 17

healthy young men [69]. During the first visit, anthropometrics were collected and optimal walking speed was determined and VO_{2MAX} was determined. On the following visits, participants walked at their optimal walking speed with and without BFR while collecting data with a metabolic cart. These trials consisted of five bouts of 3 minutes of walking with 1 minute of rest in between sets. Blood flow was restricted using a 6 cm wide that was inflated to a final pressure of 200 mmHg; this pressure was maintained throughout the walking trials, including during the rest periods. Prior to the BFR and non-BFR walking, resting VO_2, minute ventilation, heart rate and RER were collected, followed by a 20-minute quiet rest. Participants then completed 5 sets of 3 minutes of treadmill walking at a Froude number of 0.25 with 1 minute of standing rest in between bouts. The mean of the last minute of each walking bout was used as steady state data. Walking economy was also determined ($mL \cdot kg^{-1} \cdot km^{-1}$). Following completion of the exercise bouts, participants were seated for a 30-minute rest during which metabolic data were recorded. The termination of excessive post-exercise oxygen consumption (EPOC) was identified as when resting VO_2 was within 1 standard deviation of resting VO_2 for two straight minutes. The duration and area under the curve for EPOC were then determined and converted to an energy equivalent. Partial and cumulative oxygen deficits were also calculated. Data were analyzed using a two-way repeated measures ANOVA (condition * time) with Bonferroni's correction for post-hoc tests. Oxygen deficit was significant increased ($p<0.05$, 1679.4±250.5 mL vs. 1942.2±396.1 mL in the BFR group), as was the magnitude of EPOC ($p<0.05$ for both, 720.6±278.9 mL vs. 917.1±323.0 mL or 3.6±1.4 kcal vs. 4.6±1.6 kcal) although the length of EPOC remained similar (3.8±2.4 min vs. 4.4±2.2 min). Walking economy, relative intensity (%

of VO_{2MAX}), ventilation, and HR were higher in the BFR group during each walking bout (p<0.05 for all). The authors concluded that BFR walking resulted in a greater magnitude of EPOC and this difference is partially explained by the effects of BFR on relative intensity and cumulative oxygen deficit [69].

EPOC AND RESISTANCE TRAINING

Bloomer compared energy expenditure and other physiological variable between short bouts of resistance and aerobic exercise controlled for duration and intensity in 10 young males [70]. Participants performed a squat 1RM and a VO_{2MAX} on a cycle ergometer prior to testing. The testing sessions consisted of 30 minutes of squatting at 70% 1RM and aerobic cycling at 70% of VO_{2MAX}. The back squat session consisted of repetitions to volitional fatigue (5-12 reps) separated by 1.5-2 minutes of rest. Metabolic data were collected during exercise. Differences between exercises were assessed using one-way ANOVAs. Mean intensity between groups varied slightly (mean VO_2 73.06±0.50%, %1RM 61.81±1.58%). Total VO_2 was significantly higher with cycling (87.51±3.39 L vs. 53.31±2.61 L, p<0.0001), while the mean HR was not significantly different (p=0.99). However, mean RER was significantly higher with squatting (1.03±0.01 vs 0.94±0.01, p<0.0001), as was RPE (16.96±0.41 vs 14.88±0.42, p=0.0024). The author concluded that multi-joint resistance exercise – such as the back squat using a hypertrophy training protocol – can allow for an energy expenditure great enough to meet ACSM guidelines for physical activity [70].

Farinatti et al. evaluated the differences in energy expenditure and substrate utilization following resistance exercise using either chest flies or leg press in 10 resistance-trained males [72]. Participants were tested on four non-consecutive days.

On the first and second days, anthropometrics, resting metabolic data, and 15RM for leg press and chest fly were collected. On the third and fourth day the participants performed one of the two exercises for 5 sets of 10 reps at ~60% 1RM in a counterbalanced fashion. Metabolic data were collected before, during, and for 90 minutes of recovery. These data were used to determine EPOC, energy expenditure, and respiratory exchange ratio. Repeated measures ANOVAs with Fisher post hoc tests were used to test differences between exercises and over time. The leg press had a significantly greater VO_2 during exercise (6.54±1.26 L vs 3.31±0.71 L, p=0.006) and greater EPOC until four minutes post-exercise (p<0.001). The duration of the EPOC was not affected by the type of exercise. Approximately 45% of net EPOC occurred prior to 5 minutes while the remaining 55% occurred between 10 and 90 minutes. Total EPOC was significantly higher with leg press compared to chest flies after 40 minutes of recovery (7.36±1.10 L vs. 4.73±0.99 L, p<0.001). As determined by changes in the RER value during recovery, there were no significant differences in carbohydrate consumption between exercises (p=0.62) while fat consumption was slightly higher with leg press compared to chest flies (p<0.01). The authors concluded that while EPOC duration was similar, the total VO_2 and RER were influenced by the exercised muscle mass and were thus greater with leg press than chest flies [72].

Elliot et al. investigated the effects of different types of weight training protocols on EPOC in four men and five women [71]. Participants were randomly assigned to one of four exercise protocols for each session: 40 minutes of cycling, 40 minutes of circuit training, 40 minutes of heavy resistance exercise and control. Participants completed a 1RM for eight exercises prior to the study: bench press, knee extension, leg curl,

seated leg press, lat pulldown, military press, seated row and seated chest fly. The cycling protocol consisted of cycling at 75% of max HR; the low-resistance circuit consisted of four sets of 15 reps at 50% 1RM with 30 seconds of rest in between sets. The heavy resistance workout was performed at 80-90% of 1RM for repetitions to volitional fatigue (3-8 reps) with one to two minutes in between sets. Oxygen consumption, carbon dioxide production, ventilation and heart rate were recorded during and after exercise to determine EPOC. Repeated measures ANOVA were used to determine differences between groups. During exercise, cycling and circuit weight training burned significantly more calories than heavy resistance exercise ($p<0.01$). Following exercise, both the heavy resistance and circuit weight lifting had a significantly higher EPOC than cycling ($p<0.05$ for both). The authors concluded that exercising muscle mass is most likely directly proportional to EPOC and that resistance training can create a similar amount of EPOC to aerobic exercise [71].

Vianna et al. compared EPOC and HR following different resistance training exercise in 14 resistance trained males [73]. During the first and second visits, participants had their anthropomorphic measurements taken (height, body mass, and completed a 1RM for bench press, half-squat, pull-down, and triceps pushdown. During the third and fourth sessions participants performed the exercises in a random order at 80% 1RM. Oxygen consumption, carbon dioxide production, ventilation, and HR were measured during exercise and for 5 minutes after exercise as the participant sat quietly. Data were averaged every 10 seconds. The time recovery constant was determined using a mono-exponential curve and represented the time it took to reach 63% of peak oxygen consumption and the fast component of EPOC was calculated. The highest

VO_{2Peak} values were seen following the half-squat, and then the pull-down and triceps push down, which were all significantly higher than that of the bench press (p<0.05). For all exercises, the VO_2 had returned to pre-exercise levels by the end of the 5-minute period (p=0.99). The EPOC was significantly higher for the half-squat compared to the bench press and triceps pushdown (2.10±0.59 L vs. 1.09±0.41 L and 1.23±0.32 L respectively, p<0.05), but was not significantly different from the pull down (1.50±0.80 L). The decline of VO_2 following exercise remained similar between protocols. The peak HR did not vary between exercises but following exercise the HR decreased significantly more after the half-squat exercise compared to the other three exercises (p<0.05). The authors concluded that muscle mass involved in resistance exercise was directly proportional to EPOC and inversely proportional to vagal reactivation [73].

While oxygen consumption during resistance training is often less than that of an aerobic activity of similar intensity [70], the EPOC is often greater [71], especially in activities utilizing a larger muscle mass, such as the back squat [72, 73]. Blood flow restriction with walking appears to increase the net cost and relative intensity of the exercise [68] and causes the participants to breathe harder as well as causing an increase in EPOC that is partially due to an increase in cumulative oxygen debt [68, 69]. It is unknown if this effect is present with resistance exercises performed with BFR.

Acute BFR with Resistance Exercise

Blood Flow Restriction is most often paired with resistance exercise and has become increasingly popular in recent years. However, while there are several different commonly used protocols, a truly standardized protocol has yet to emerge due to

differences in the exercise prescription (set reps vs. reps to failure), the method of BFR, and amount of occlusive pressure utilized.

Fry et al. investigated the effects of an acute bout of BFR training on mTORC1 and muscle protein synthesis in seven older males [6]. Participants randomly completed either a BFR or control exercise session first followed two weeks later by the other protocol. The BFR group completed one set of 30 bilateral leg extensions at 20% of 1RM, followed by three more sets of 15 reps with 30 seconds between sets, while blood flow was restricted using a KAATSU cuff at 200 mmHg. The control group completed the same exercise without BFR. Blood was collected pre- and several times post-exercise as well as muscle biopsies from the vastus lateralis pre- and post-exercise. Blood lactate, cortisol, growth hormone, and glucose were then analyzed. Oxygen saturation was monitored throughout exercise. Repeated measures ANOVAs were used for comparison between groups and over time. Lactate increased significantly in both conditions during exercise and remained elevated for 45 minutes after exercise and was significantly higher in the BFR condition compared to control at 30 minutes post exercise ($p<0.05$). Cortisol increased significantly in BFR condition over time and compared to controls for 2 and 1.5 hours respectively ($p<0.05$ for both). Growth hormone also increased significantly in the BFR condition over time and compared to the control condition ($p<0.05$ for both). Leg circumference increased 2.5±0.6 cm in the BFR condition and remained elevated for 30 minutes post exercise ($p<0.05$) and was significantly greater than the control condition (1.3±0.3 cm, $p<0.05$). Oxygen saturation decreased to 74.5±4.8% in the BFR condition compared to 91.1±1.0% with control ($p<0.05$). Muscle protein synthesis was significantly increased

with BFR 3 hours post exercise compared to the control condition (56%, $p<0.05$). The BFR group also had a significant increase in the phosphorylation of S6K1 and rpS6 compared to controls at 1 and 3 hours post exercise, indicating an increase in the mTORC1 signaling pathway, most likely through improved translation initiation and ribosomal biogenesis. Markers of the MAPK signaling pathway were also increased 1 hour after exercise compared to the control condition ($p<0.05$). Ratings of perceived exertion were collected and exercise with BFR was reported as being comparable to high-intensity resistance exercise (7-8 out of 10). The authors concluded that a single bout of low resistance exercise with BFR stimulates muscle protein synthesis and enhances the mTORC1 and MAPK signaling pathways [6].

Cook et al. (2007) investigated the acute effects of eight different BFR protocols on fatigue of skeletal muscles and compare them against the current ACSM strength training recommendations [102]. Twenty-one recreationally active individuals with normal BP and no blood clotting disorders or lower extremity injury participated in five separate visits. Nine protocols (two per session, one per leg) were completed in a counterbalanced order. Isometric muscle strength was assessed at 60 degrees of knee flexion before and after the exercise protocols. Participants performed leg extensions with a 2 sec eccentric and concentric phase. The BFR protocols used either 20 or 40% of MVC, partial (30% greater than SBP) or complete (300 mmHg) occlusion, and either intermittent or continuous pressure. All acute bouts of BFR exercise were more fatiguing than standard high intensity exercise and had greater force decrements post exercise (high intensity 19%, BFR 24-33%). The 20% MVC with continuous partial occlusion was the combination recommended by the authors since it was the only one to create

significantly greater force decrements in MVC compared to control and all subjects responded to it rather than the variability in fatigue seen in 20% MVC with continuous complete occlusion. The authors also anecdotally observed the 20% MVC with continuous partial occlusion condition was more comfortable and tolerable than the other conditions. All BFR conditions created at much fatigue as control, but only 20% MVC with continuous partial occlusion created significantly more fatigue than control and could thus stimulate muscle growth [102].

Madarame et al. (2010b) also measured markers of clot formation to investigate the effects of low-intensity BFR on coagulation systems in 10 male participants [103]. Participants performed 4 sets of leg press exercise at 30% of 1RM (30+15+15+15 repetitions) with 1 minute between sets while BFR was applied to the most proximal portion of their thighs using a 150-160 mmHg of pressure. Blood samples were collected before, 10 minutes post-exercise, as well as 1, 4, and 24 hours post exercise and analyzed for hemoglobin, hematocrit, prothrombin fragment 1+2, and other markers of coagulation. Repeated measures ANOVAs were used to examine differences. Reduction in plasma volume was significantly greater with BFR that without (-4.6±3.5% vs. 0.9±3.7%, $p<0.05$). There were no other significant changes in markers of coagulation. The authors concluded that four sets of low-intensity leg press with BFR did not significantly change markers of thrombin generation or markers of intravascular clot formation [103].

Suga et al. (2010) investigated the dose effects of differing exercise intensity and restrictive pressures during BFR in twelve (6 men/6 women) healthy young subjects [104]. Participants performed right ankle plantar flexion; each condition had 3 sets of 30

repetitions of lifting a weight 5 centimeters. Two conditions were performed without BFR: low intensity (20% 1RM) and high intensity (65% of 1RM). The four conditions with BFR were performed at 20, 30, or 40% of 1RM with moderate pressure (130% of SBP), or 20% of 1RM with high pressure (200 mmHg). The cuff used for BFR was 18.5 cm wide. Magnetic resonance spectroscopy was used to assess PCr, P_i, phosphate, and pH before and after exercise. Phosphocreatine levels significantly decreased, and phosphate levels significantly increased following exercise in all conditions (p<0.001 for both). Intramuscular pH significantly decreased (p<0.05) in all but the low intensity condition. Intramuscular metabolites and pH during 20% 1RM with BFR were significantly higher than those following low intensity exercise without BFR (p<0.001) but significantly lower than those following high intensity exercise without BFR (p<0.05). Fast-twitch muscle fibers were recruited in all BFR conditions and high intensity exercise without BFR but not low-intensity exercise without BFR with the highest recruitment occurring during the 40% of 1RM with moderate pressure. This condition also had greater metabolic stress than the high-intensity exercise without BFR. Using BFR with 30% 1RM intensity created similar metabolic stress to high-intensity exercise without BFR, this condition was suggested by the authors for further use in BFR research [104].

Yasuda et al. (2010a) investigated the changes in venous blood flow and metabolite levels with low-intensity BFR exercise compared to traditional high-intensity training in 16 young males [105]. Eight participants were used to investigate changes in venous blood flow while the remaining eight were used to investigate venous blood gasses and metabolites. The venous blood flow group performed three sets unilateral

elbow curls at 20% of 1RM with three separate pressures (no pressure, 30 mm cuff inflated to 100 mmHg, 30 mm cuff inflated to 160 mmHg). Blood flow was measured prior to, immediately after and 15 minutes after exercise. Blood samples were collected at similar time points. Blood flow was assessed via Doppler ultrasound. For the assessment of venous blood gasses and metabolites, the remaining eight participants performed biceps curls for three trials at 20% 1RM with three separate pressures (no pressure, cuff inflated to 100 mmHg, cuff inflated to 160 mmHg) and one trial at 70% 1RM without BFR. Blood samples were collected prior to exercise, during exercise and immediately after, as well as after a 15-minute rest and analyzed for venous PO_2, P_vCO_2, S_VO_2, pH_v, hematocrit, blood glucose and blood lactate. Repeated measures ANOVA were used to analyze differences. For the blood flow group, blood flow decreased with the application of BFR (56% of baseline for 100 mmHg, 39% of baseline for 160 mmHg), after exercise blood flow increased to 164% and 392% of pre baseline for 100 mmHg and 160 mmHg respectively. The P_vO_2 decreased significantly with BFR but not control. The decrease in S_vO_2 was significantly greater with 160 mmHg vs. 100 mmHg and both were greater than controls. The levels of P_vCO_2 increases with all trials but was higher with BFR than controls. The blood pH_v was decreased during and after exercise, and these changes were greater with BFR compared to controls. Hematocrit and blood glucose did not change with BFR. Changes in blood lactate were significantly higher with 160 mmHg of BFR compared to both 100 mmHg of BFR and controls. The authors believe that the changes observed with pH_v, P_vO_2, and lactate concentration appear to be large enough to stimulate group III and IV sympathetic afferent nerve fibers. They concluded that the muscle venous blood gas and metabolite

changes create different environments in low-intensity BFR exercise and high-intensity resistance exercise [105].

Karabulut et al. (2011) investigated the effects of several initial pressures of BFR on tissue oxygenation and venous return in six supine young males [106]. They used a 50 mm wide KAATSU cuff to inflate to an initial pressure of 35 to 45 mmHg increasing to 140 to 160 mmHg, 45 to 55 mmHg increasing to 160 to 180 mmHg, or 55 to 65 mmHg increasing to 180 to 220 mmHg; oxygen saturation of the quadriceps muscle was assessed in each condition. The initial restrictive pressure used for KAATSU BFR affected the amount of venous return and tissue oxygenation during exercise, thus varying the blood pooling and metabolite accumulation that occurs which may be a contributing factor for the mixed responses to BFR in other studies [106].

Loenneke et al. (2012c) also investigated time under tension, lactate and HR responses in knee extensions to failure with BFR. Thirteen recreationally active individuals performed knee extensions to fatigue using a 1 sec concentric, 1 sec eccentric protocol and performed repetitions to failure with and without BFR. Participants performed two sets to failure with 30 seconds of rest between sets. Work was calculated as the number of repetitions time the mass of resistance. Blood lactate was measured prior to exercise, immediately after and 3 and 5 minutes after the second set. Heart rate was measured prior to and immediately after exercise as well as 3 and 5 minutes post exercise. Total work was significantly lower with BFR for the first ($p<0.05$, BFR 520.89±212.76 vs control 689.13±213.06 kg, ES 0.79) and second ($p=0.001$, BFR 115.56±73.81 vs control 281.03±114.5 kg, ES 1.65). Blood lactate levels were also significantly difference between BFR and control immediately after ($p=0.001$, BFR

2.7±1.4 mmol·L^{-1}, control 4.8±1.4 mmol·L^{-1}, Cohen's D -1.2) and at 3 min post (p=0.023, BFR 5.0±2.2 mmol·L^{-1}, control 6.8±1.5 mmol·L^{-1}, Cohen's D -0.7). There were no significant differences in HR. While the control group performed more work, blood lactate levels were fairly similar between conditions. In conclusion, pBFR reduced the number of repetitions to failure compared to exercise without BFR and could potentially be used for those who cannot exercise at that load for extended periods or complete that amount of work. [107]

Suga et al. (2012) also investigated the responses in metabolic stress and fast twitch muscle fiber recruitment during sets of low intensity BFR using continuous or intermittent pressure and compared these results to those during traditional exercise in twelve young males [108]. Participants performed right ankle plantar flexion on two different days with two conditions each day. Each condition had 3 sets of 30 reps lifting a weight 5 centimeters above the ground. Two conditions were performed without BFR: low intensity (20% 1RM) and high intensity (65% of 1RM). The two conditions with BFR were performed at 20% 1RM using either intermittent or continuous pressure. The pressure used for BFR was 130% of SBP using an 18.5 cm wide cuff. Inorganic phosphate, intramuscular pH, and phosphocreatine levels were assessed before and after each condition. Intramuscular metabolites were significantly changed compared with resting values post exercise ($p<0.001$), intramuscular pH also decreased significantly in both BFR conditions and the high-intensity condition ($p<0.001$). The change in total PCr was significantly greater with continuous BFR as opposed to intermittent BFR ($p<0.001$). It was concluded that multiple set low intensity BFR with

continuous venous occlusion created intramuscular metabolic stress and skeletal fiber recruitment similar to traditional high intensity exercise [108].

Wernbom et al. investigated neuromuscular fatigue and recovery and fiber morphology after a single session of low-load resistance exercise with and without BFR in eight male and four female participants [109]. One week prior to testing, participants performed 1RM testing for unilateral knee extension and were then familiarized with the equipment to be used in the study. Participants performed maximal voluntary contraction (MVC) for EMG testing and also underwent neuromuscular electrical stimulation (NMES). Following a 5-minute rest, participants performed five sets of knee extensions at 30% 1RM with BFR (135 mm wide cuff inflated to 90 mmHg for females and 100 mmHG for males) to failure with 45 seconds between sets. Two additional MVC tests were performed at one and two minutes post exercise as well as NMES testing at three minutes post exercise. The same protocol without BFR was then repeated for the contralateral leg following a 10-minute rest. Muscle biopsies were collected from the vastus lateralis 1, 24, and 48 hrs post exercise for each leg and assessed for muscle cell damage. There were significantly larger reductions in MVC at one and two minutes post exercise in the BFR leg compared to the non-BFR leg. This decline lasted at least 48 hours, especially in the BFR leg. Additionally, tetranectin levels were increased in both legs (but especially the BFR leg) following exercise. The authors believe that the acute fatigue was most likely due to metabolic stress from exercise with BFR. The longer lasting force decrements were most likely due to muscle damage that occurred with exercise, as evidenced by the increases levels of tetranectin. They then concluded that the BFR increased the susceptibility of the working muscles to

damage from lower intensities then are normally required. This may be the reason for Type 1 muscle fibers showing more damage than Type II fibers since they were subjected to stress longer. The torque decrements and increased muscle membrane leakiness indicate that there is a potential for muscle damage following a first-time bout of BFR using multiple sets of all-out effort [109].

Cook et al. (2013a) compared endurance, torque decrement, central activation, muscle activated and evoked contractile function after acute bouts of leg extension performed at low intensity, high intensity, and low intensity with BFR in eight healthy active males. Participants completed three sets of knee extensions to volitional fatigue with 30 seconds of rest in between sets. Exercise volume, decrements in peak isometric torque, RPE, HR, central activation, contractile function, and EMG were measured. Low intensity exercise was performed at 20% of peak torque and high intensity exercise was performed at 70% of peak torque with a two second concentric/eccentric cadence. Blood flow restriction was maintained throughout the LL with BFR condition and was performed using a 5 cm wide KAATSU cuff at about 1.3 to 1.5 times SBP. Participants performed significantly more repetitions in the low intensity and low intensity with BFR conditions than the high intensity condition ($p<0.01$, partial eta squared 0.48); however, exercise volume was not significantly different between groups ($p=0.07$, partial eta squared 0.27). Similarly, isometric peak torque decreased after all exercises ($p=0.004$, partial eta squared 0.63), but was not significantly different between exercises ($p=0.26$, partial eta squared 0.18). All conditions created a 19% increase in EMG activity of the quadriceps. High intensity exercise had higher EMG activity than low intensity ($p=0.02$) and low intensity with BFR ($p=0.04$). There were no

differences between conditions for HR and RPE but there was a significant difference over time for HR (p<0.01, partial eta squared 0.65). The authors concluded that all exercise conditions resulted in similar torque decrements despite the increased volume with the low intensity and low intensity with BFR conditions. Additionally, they concluded that muscular fatigue was primarily due to peripheral factors rather than changes in central activation [65].

Fitschen et al. compared the effects of continuous or intermittent resistance exercise with BFR on perceptions of pain during repetitions to failure during a single session of exercise in 11 healthy participants [110]. This was followed by five weeks of intermittent or continuous BFR resistance to examine the effects on skeletal muscle size and strength in 32 healthy participants. Quadriceps and hamstring isokinetic muscle strength was tested in the non-dominant leg at baseline, immediately post, and 24 hours post training. Testing was performed at $60°\cdot s^{-1}$ for two sets of six repetitions with 3 minutes between sets. Participants then performed repetitions to failure for four sets with ninety seconds rest between sets with either control (no pressure), intermittent BFR (restriction only during exercise), or continuous BFR. A 50 mm wide cuff inflated to 160 mmHg was placed on the proximal thigh prior to exercise. Pain was measured immediately after each set on a scale of 1-10. The training study evaluated bilateral quadriceps and hamstring muscle strength in the same manner as the acute study, as well as 1RM for leg press, leg extension, and hamstring curl and body fat as determined by DEXA. Participants were randomly assigned to perform either continuous BRF exercise, intermittent BFR exercise, or non BFR exercise. Training was conducted thrice weekly and consisted of leg press, leg extensions and seated hamstring curls at

30% of 1RM and bodyweight standing calf raises. Participants built up to 4 sets of 30-15-15-15 reps over the course of 4 training sessions and increased to 30-20-20-20 during the last two weeks of training. Differences over time and between conditions was assessed using repeated measures ANOVA with a Tukey post-hoc test. In the acute study, pain differed significantly between conditions during sets 3 (p=0.036) and 4 (p=0.010), with continuous BFR being significantly greater than intermittent BFR (~4.5 vs. ~6.25) and control (~4.75 vs ~6.25 or ~7.0 for sets 3 and 4). As expected, significantly more repetitions were performed during the control condition (61±12 control vs 51±12 intermittent and 50±9 continuous). Maximum voluntary contraction was significantly reduced immediately post exercise (p=0.002) and 24 hours post-exercise (p=0.006). For the training study, maximal isokinetic strength during knee extension increased by 5.2±10.41% (p=0.033) and knee flexion increased by 6.4±11.5% (p=0.007). However, there was no significant differences between groups or over time for both knee extension (p=0.908) or knee flexion (p=0.633). Maximum pain reported decreased from 4.36±2.34 to 3.4±2.12 with training (p=0.005), but did not differ between groups over time. The authors concluded that intermittent BFR may be a more comfortable alternative to continuous BFR exercise since they resulted in similar levels of fatigue and that 5 weeks of low-load resistance training with BFR increases muscled strength in a similar manner to low-low load resistance training without BFR [110].

Karabulut et al. (2014) examined the effects of different initial pressures for BFR and low-intensity resistance exercise on tissue oxygenation, oxygenated and deoxygenated hemoglobin, leg strength and blood lactate response in 20 male participants [111]. Isometric maximal voluntary contraction torque was collected both

before and after the knee extension exercises. Participants completed four sets (30+15+15+15) of knee extensions at 20% of 1RM with BFR (5.5 cm KAATSU cuff) at an initial pressure of either 40-45 or 60-65 mmHg prior to full inflation (144% of SBP). Blood lactate concentrations were collected prior to, during, and 0, 5, 10, and 20 minutes post exercise. Tissue oxygen saturation was recorded prior to cuff placement, during exercise, and the 3 minutes post exercise. Repeated measures ANOVAs were used to assess differences in variable over time and between pressures. There were no significant differences in lactate concentrations between groups. Tissue oxygenation decreased during exercise in both groups (p<0.001), but significantly more so with an initial pressure of 60-65 mmHg (p=0.01). A significant condition by time interaction effect existed as well (p=0.02). There were no differences between initial pressures for oxygenated hemoglobin, but there was a significant difference in deoxygenated hemoglobin (p=0.02). They authors concluded that increased initial restrictive pressures resulted in decreased tissue oxygenation, and increased blood and deoxyhemoglobin accumulation as well as muscular fatigue, which could have an effect on the desired training response [111].

Loenneke et al. (2012) examined the effects of different levels of BFR while exercising at 20% of 1RM on torque, muscular activation and neuromuscular fatigue in 14 male participants [3]. Three separate levels of arterial occlusion were used: 40%, 60%, and 80%. Occlusion was maintained using a 13 cm wide pneumatic cuff applied to the most proximal portion of the right thigh. Maximal voluntary contractions (MVC) and 20% MVC with and without BFR were performed before and after exercise. The BFR exercise consisted of knee extensions performed at 20% of 1RM (4 sets,

30+15+15+15 reps, 30 seconds rest between sets) at a 1-second concentric-eccentric pace. Repeated measures ANOVAs were used to assess differences over time and between occlusive levels. Torque was decreased significantly (5.2%, $p<0.01$) post exercise only for 80% occlusion, no other significant differences existed. Higher occlusive pressures led to greater decreases in muscle activation as indicated by decreases in median frequency for the vastus lateralis and rectus femoris. The authors concluded that the magnitude of neuromuscular fatigue is dependent on the levels of vascular restriction. They also stated that BFR should not be prescribed using a standardized pressure, but rather a percentage of the pressure at which arterial occlusion occurs.

Yasuda et al. (2014) investigated the effects of BFR when performed with elastic band resistance on muscle activation in nine adult males with resistance training experience [112]. Participant performed biceps curls and triceps extensions against elastic bands with and without BFR in two separate sessions one week apart. A 30 mm (KAATSU) wide cuff was inflated to 170-260 mmHg to restrict blood flow. Muscle activation was assessed using surface EMG. Blood lactate concentrations were assessed at baseline, and immediately after and 15 minutes after exercise. Heart rate was assessed before and after exercise and RPE were assessed immediately after each exercise. A two-way repeated measures ANOVA was used to assess differences between conditions and over time. Triceps iEMG increased during BFR (~46%) and was significantly greater than control during the last set ($p<0.05$). Bicep flexion with BFR increased iEMG (~69%) and was greater than control during the second to last set ($p<0.05$). There were no significant increases in control during triceps extension and

biceps flexion. Blood lactate was significantly higher following BFR compared to control (3.6 vs. 2.1 mmol·L^{-1}, p<0.05). Blood lactate also remained significantly higher with BFR at 15 minutes post exercise (1.8 vs. 1.2 mmol·L^{-1}, p<0.05). Heart rate and RPE were greater following BFR (p<0.05 for both). The authors concluded that low-intensity, resistance exercise with elastic bands performed with BFR enhances muscle activation in the upper extremity [112].

Neto et al. compared the hypotensive effects of resistance exercise with and without BFR in 24 male participants [113]. Four separate experimental protocols were performed: high intensity (four sets of 8 repetitions at 80% 1RM with 2 minutes between sets) resistance training, low-intensity (one set of 30 repetitions followed by three sets of 15 repetitions at 20% 1RM with 30 seconds between sets) resistance training, low-intensity (one set of 30 repetitions followed by three sets of 15 repetitions at 20% 1RM with 30 seconds between sets) resistance training with BFR, and control (seated rest for 30 minutes). Participants performed biceps curls, triceps extensions, knee extensions and knee flexion with 1 minute of rest in between sets. Blood pressure was assessed before and after each exercise session and mean arterial pressure was calculated. Blood flow restriction was achieved using a 60 mm wide cuff for the arms and a 100 mm wide cuff for the legs inflated to 80% of the occlusive pressure for that limb and deflated for 30 seconds in between sets. Repeated measures ANOVAs were used to assess differences between conditions. There were no significant differences in SBP between protocols (p>0.05). Significant differences were found in DBP between high-intensity, low-intensity, and low-intensity with BFR vs controls (p=0.034, 0.009, 0.012 respectively). Significant differences were found in Mean BP between high

intensity vs. controls, low-intensity vs. controls, and low-intensity with BFR vs. controls (p=0.001, 0.010, and 0.004, respectively) immediately post-exercise. Total work was significantly greater in the high-intensity protocol compared to the low-intensity and low-intensity with BFR protocols. The low-intensity with BFR protocol promoted a faster and longer-lasting DBP response than other protocols. The high-intensity and low-intensity with BFR also showed a reduction in Mean BP at 30 and 60 minutes post-exercise, although the low-intensity with BFR protocol did not show this response immediately following exercise. The authors concluded that both high-intensity and low-intensity with BFR can be used to maximize the hypotensive effects of resistance training, while low-intensity with BFR can also be used to improve DBP and Mean BP [113].

Yasuda et al. (2015a) investigated the effects of low-intensity resistance exercises to fatigue with and without BFR on muscle swelling in 10 male participants [114]. The exercises consisted of four sets of paced unilateral elbow flexion at 20% of 1RM to exhaustion with either three minutes (control) or 30 seconds (BFR) between sets. For the BFR exercises, the cuff (30 mm, KAATSU) was positioned at the most proximal portion of the arm and inflated to 160 mmHg. Ratings of perceived exertion were collected every 10 repetitions and at the end of each set. Muscle thickness was assessed via ultrasound before, between sets, and 0, 15, 30, and 60 minutes post-exercise. Muscular activity was recorded via EMG throughout the exercise as was heart rate. Blood samples were taken prior to, and 0, 15, 30, and 60 minutes post-exercise and analyzed for hematocrit, lactate concentration and creatine kinase. Repeated measures ANOVAs were used to assess differences in between groups. As expected, the control group completed a greater number of repetitions during the first

and second sets, as well overall (p<0.01 for all). Muscle thickness increased significantly in both conditions during recovery (control 120% and BFR 121% of baseline, p<0.01). Lactate concentration and hematocrit were significantly increased in both conditions immediately after exercise (p<0.01), but not different between groups. There were no differences between groups in integrated EMG activity. The increased muscle swelling following exercise and increased muscle activity did not differ between conditions and followed a similar time course. The authors concluded that low-intensity resistance exercise to volitional fatigue promotes muscles swelling regardless of BFR and is maintained even 15 to 60 minutes after exercise.

Loenneke et al. (2016) investigated the differences in perceptual responses to six different levels of arterial occlusion (40-90% of occlusion) within 14 participants (10 male, 4 female) [115]. A 5 cm wide cuff was applied to the most proximal portion of the arm and the cuff was inflated to 40, 50, 60, 70, 80, or 90% of the occlusive pressure. The order and arm used for each trial was randomized. Exercise bouts consisted of one set of 30 repetitions of elbow flexion at 30% of 1RM followed by three sets of 15 repetitions with 30 seconds of rest in between sets. Ratings of perceived exertion and ratings of discomfort were collected prior to that start of exercise and the conclusion of each set. Differences between trials were assessed using Friedman non-parametric and Wilcoxon related samples tests. Ratings of perceived exertion and ratings of discomfort were not driven by the absolute or relative level of BFR. There was significant a significant interaction between the pressure applied and number of repetitions completed (p<0.001) as less repetitions were completed in the later sets when using a greater degree of occlusion. However, this was not found to be related to

the perceptual response. The authors concluded that the perceptual response was not linked to pressures high enough to cause venous occlusion but low enough to permit arterial flow. Thus, the perceptual rating of the individual is unlikely to be a limiting factor when using BFR, although higher pressures decreased the ability of the individual to perform the activity [115].

Loenneke et al. (2011) have also discussed the potential safety issues with BFR training. The authors concluded that peripheral blood flow response is similar between BFR and traditional resistance training. Exercise with BFR does seem to promote capillarization, no changes in arterial stiffness, and generally peripheral blood flow responds similary to traditional exercise. The risk of increased coagulation activity did not appear to be associated with BFR exercise and only caused minimal muscle damage without having an effect on nerve conduction velocity. The authors concluded that BFR training conducted in a controlled environment by trained and experienced personnel is a safe training alternative for individuals regardless of age and training status [75].

Nakajima et al. (2006) investigated the use and safety of KAATSU BFR training in 105 facilities [98]. Responses were obtained from 105 of 195 facilities. Side effects of KAATSU BFR training were reported as subcutaneous hemorrhaging (n=1651, 13.1%), temporary numbness (n=164, 1.297%), cerebra anemia (n=35, 0.277%), cold feeling (n=16, 0.127%), venous thrombus (n=7, 0.055%), pulmonary embolism (n=1, 0.008%), rhabdomyolysis (n=1, 0.008%), and deterioration of ischemic heart disease (n=2, 0.016%). Fainting was also reported in rare cases. The authors concluded that

the rate of severe side effects was low and that BFR training is safe for both healthy individuals and those with various disease states if applied correctly [98].

Nakajima et al. (2011) also discussed the key safety considerations with KAATSU BFR training [76]. The authors suggested conforming to the following principles when conducting BFR training: 1) no normal exercise contra-indications, 2) no hemodynamically unstable patients, 3) no patients with thrombotic disease, 4) inform patients of the possibility of petechial hemorrhaging and numbness that may occur with BFR, 5) adapt training to the individual and apply appropriate as well as safe restrictive pressures, 6) monitor for presyncope and fainting, 7) avoid conducting BFR training for longer than 10 to 15 minutes for the upper limbs and 15 to 20 minutes for the lower limbs, 8) ensure blood pressure is less than 160/95 mmHg, 9) do not conduct training when the individual is ill [76].

Low intensity resistance exercise with BFR has been shown to have similar post-exercise hypotensive effects to high intensity resistance exercise [76] as well as similar deficits in neuromuscular activation following exercise [102] although the intermuscular environments differ [105]. Increases in the mTORC1 and MAPK pathways and activation of Type III and IV muscle afferent fibers have been reported following an acute session of low intensity resistance training with BFR [6, 105]. The intensity of the exercise seems to be increased when BFR is applied [59, 107, 115], leading to increased fatigue (less repetitions to failure [102, 111]. This has been shown to be related to the restrictive pressure used, with higher pressures leading to a quicker onset of fatigue [7, 106, 111]. Increased muscular activation has also been reported when using BFR [60, 112]. However, some debate remains about the effectiveness of BFR,

since exercise to fatigue has been shown to cause acute muscle hypertrophy regardless of the use of BFR [114] and there is conflicting research between whether continuous (19) or intermittent BFR [110] is the most beneficial and comfortable for participants. Suga et al. suggests that at least 30% of 1RM be used to increase metabolic stress [104]. However, resistance training performed with BFR is still considered safe, with a low risk for severe side effects, as long as certain precautions are followed [75, 76, 98, 103].

Oxidative Stress with BFR

Oxidative stress has often been put forth as one of the potential mechanisms for muscular hypertrophy when using BFR [12, 13]. However, one review article on BFR by Pope et al. [13] stated that the topic of oxidative stress "is obviously complicated, and there remain large gaps in our knowledge base at this time."

Takarada et al. investigated the effects of low intensity BFR exercise on growth hormone and norepinephrine as well as other hormonal markers in six male participants. The exercise protocol consisted of five sets of bilateral knee extension at 20% of 1RM to failure with 30 seconds of rest between sets. The mean repetitions per set were 14.4±1.6 reps. Cuffs were placed at the proximal thigh (33 mm, mean pressure 214±7.7 mmHg) and inflated throughout exercise. A control session was also completed without BFR. Blood was collected 5 minutes prior to exercise and 0, 15, 45, and 90 minutes post-exercise and analyzed for lactate, growth hormone, norepinephrine, IL-6, lipid peroxide and creatine phosphokinase. EMG recordings were collected from the vastus lateralis. Repeated measures ANOVAs were used to assess differences. All concentrations of lactate growth hormone and norepinephrine increased

significantly with BFR exercise but not with control immediately post exercise (p<0.05), but were no different after 30 minutes. The time courses for all three were similar following exercise. The levels of IL-6 with BFR were significantly higher than controls from 30 minutes to 24 hours post-exercise (p<0.05). Creatine kinase with BFR was slightly increased over controls over 24 hours post exercise, but not significantly so. There were no differences in lipid peroxides between protocols. Even though the workloads were similar between protocols, the relative EMG was ~1.8 times greater with BFR than without. The authors theorized that the changes in lactate concentration were tied to the local hypoxia created with BFR which could stimulate group III and IV afferent sympathetic fibers, which has been linked to growth hormone secretion. They concluded that the main intramuscular condition required to stimulate hypertrophy are acute hypoxia and the accumulation of metabolic accumulates and not oxidative stress [47].

Goldfarb et al. (2008) investigated if Blood Flow Restriction alters the oxidative stress response (protein carbonyls and glutathione) with and without low intensity resistance exercise compared to traditional moderate resistance exercise in seven resistance-trained males [46]. Body fat was determined using a three-site skinfold measurement and 1RM values for single arm biceps curl and single leg calf extension were collected during a familiarization period. Participants then performed three exercise sessions: a BFR session at 30% of 1RM, a traditional training session at 70% of 1RM, and a control session with BFR but not exercise. During the BFR session a narrow cuff was applied to the arm and inflated to 20 mmHg below SBP; the participant then completed three sets of biceps curls to exhaustion with one minute of rest between

sets. Following a five-minute rest, a similar procedure was performed for calf extensions. The traditional training session was performed in a similar fashion but without occlusion and the resistance increased to 70% of 1RM for the traditional training session. The control session followed the same methods as the BFR session but did not perform any exercise. Blood was drawn before, immediately after, and 15 minutes after exercise from the arm not involved in the testing and tested for protein carbonyls and blood glutathione ratios. Protein carbonyls were significantly elevated following the control and traditional sessions while there was not a significant change in values following the BFR session. Glutathione ratios followed a similar trend. The authors determined that BFR alone and exercise at 70% of 1RM without BFR increased oxidative stress, but exercise performed at 30% of 1RM with BFR did not [46]. However, most oxidative stress studies take samples further out that just 15 minutes, so it is unknown if changes might have occurred during the period after that.

Takarada et al. compared two low-intensity protocols (one with BFR) and found no differences while Goldfarb et al. appeared to achieve mixed results, with high-intensity and control protocols (BFR only) achieving increased oxidative stress [46, 47]. This discrepancy in oxidative stress between studies may be due to the differences in protocols; both protocols used repetitions to failure, but Takarada et al. utilized knee extensions, while Goldfarb et al. utilized bicep curls and plantarflexion exercises. A comparison between volume matched protocols utilizing multi-joint exercises such as the back squat has yet to be made.

Training Effects of Blood Flow Restriction

Blood Flow Restriction exercise training has often been suggested as an alternative training method for increasing strength in individuals who are unable to perform a traditional strength training exercise (such as the elderly and those recovering from injury or surgery). This is due to the lower training intensity used with BFR (20-30%) as opposed to traditional training which occurs at training intensities of 70% of 1RM or higher. Loenneke and Pujol have even suggested the use of BFR training for astronauts to potentially attenuate the negative effects of spaceflight [116].

Burgomaster et al. investigated the effects of low intensity resistance training on resting energy metabolites and what effect, if any, BFR would have on training induced adaptations in eight healthy men [61]. Maximal elbow flexor torque was assessed using an isokinetic dynamometer at 60° per second for both concentric and eccentric motion; 1RM was then assessed for elbow flexion. Muscle biopsies were collected from each arm before and after the training intervention. One arm was randomized for BFR (12 cm wide cuff inflated to 100 mmHg) while the other was used as a control. Participants then completed an 8-week progressive resistance training protocol at 50% 1RM. (The first two weeks consisted of two sets of 10 repetitions followed by a third to failure, then the load was increased by one set per week until six sets, which were performed for the remained of the study). Exercises were performed twice per week. The protocol was conducted with the BFR arm followed by the control arm and work was matched between arms. Post-training strength measurements were conducted on the last day of training. Muscle biopsies were assessed for muscle glycogen, ATP, phosphocreatine, and creatine. A two-factor ANOVA was used to assess differences between arms.

Muscle glycogen increased significantly more in BFR arm compared to control ($p \leq 0.05$), and the net ATP decrease was greater in BFR compared to control ($p \leq 0.05$). There were no differences in phosphocreatine or creatine following training in either arm. Elbow flexor torque increased by 9.6 and 10.5% and 1RM increased by 23 and 22% in the control and BFR arms respectively ($p \leq 0.05$); these differences were not significant. The authors theorized that the reduced rate of oxygen delivery with BFR mimicked hypoxia with caused a translocation of GLUT-4 transporters leading to increased glucose transport and thus resting glycogen concentrations. The decrease in ATP concentration may be due to the stress of chronic training or residual effects of the last training bout, although the larger net decrease in the BFR arm may be attributed to additional stress placed on the adenine nucleotide pool in that arm [61].

Sumide et al. (2009) attempted to determine the optimal compressive pressure for BFR exercise during eight weeks of training in 21 untrained males [117]. Participants were divided into four groups: no pressure, 50 mmHg BFR, 150 mmHg BFR, and 250 mmHg. Maximum isometric contraction and 1 RM were recorded before and after the training intervention. Resistance training was conducted three times a week and consisted of straight leg raises, hip abduction, and hip adduction. Ratings of perceived exertion were collected after each exercise. Paired t tests were used to examine differences within groups over time and a one-way ANOVA with Scheffe post hoc test was used to assess differences between groups. Ratings of perceived exertion decreased over the 9 weeks of training in all groups, but these changes were not significant. There was a significant increase in work performed post training in the 50 and 150 mmHg groups ($p<0.05$ and $p<0.01$ respectively. There were no changes in

muscle cross-sectional area in any group. Resistance training with BFR was shown to increase muscular strength and endurance, even at only 50 mmHg of pressure. The authors concluded that resistance exercise with relatively low BFR compression can increase muscle strength and endurance without discomfort [117].

Yasuda et al. (2010b) investigated the effects of two weeks of low-intensity BFR bench press training on muscular strength and hypertrophy in the chest and arms in 10 males [118]. Participants were divided into either a BFR or non-BFR group and performed two sessions a day, six days a week, for two weeks. Both groups completed four sets (30-15-15-15) of bench press at 30% of 1RM with 30 seconds of rest between sets. The BFR group had a cuff placed at the upper portion of the arm that was increased from 100 mmHg (day 1) to 160 mmHg (day 7). Muscle size and 1RM for bench press were assessed before and after the training intervention. Differences between and within groups were assessed with two-way repeated measures of ANOVA. Muscle thickness increased 8% in the triceps brachii and 16% in the pectoralis major with BFR training ($p<0.05$ for both) but did not change in the control group. Muscle strength also increased significantly more with BFR training compared to controls (6% vs -2%). There were no changes in growth hormone, IGF-1, IGF-BP3, CK, or myoglobin. The authors suggested that the increase in muscle strength was more closely tied to changes in muscle hypertrophy rather than neural adaptation and concluded that low-intensity bench press training with BFR increased muscular strength and size in the triceps brachii and pectoralis major [118].

Ellefsen et al. (2015) compared the effects of low intensity BFR and traditional strength training in fifteen untrained females [119]. Participants had one leg assigned to

low intensity BFR and the other was assigned to the traditional strength training group. Training was performed four days a week (two for BFR, two for traditional) for 12 weeks. Blood flow restriction was implemented using an 18 cm wide cuff inflated to 90-100 mmHg. Training consisted of five sets to failure at 30% of 1RM for BFR with 45 seconds of rest between sets and 3 sets of 6-10 reps at either 74-81% (10 reps) or 85-92% (6 reps) of 1RM with 90 seconds of rest between sets for traditional strength training. Muscle biopsies and 1 RM were recorded before and after the exercise intervention. Blood samples were collected before and after the first and last week of exercise. Muscular cross-sectional areas were measured with MRIs before and after the exercise intervention. Within group differences were determined with paired t tests and within between group differences were determined using two-way repeated measures ANOVA with Holms-Sidak post hoc. There was a 10±7% increase in knee extension strength with 12 weeks of BFR training (p<0.05) while standard strength training resulted in a 12±6% increase (p<0.05). Cross sectional area of the quadriceps (Distal Quad: BFR 6±4%, traditional 7±8%; Proximal Quad: BFR 6±4%, traditional 9±4%, difference p<0.05) and vastus lateralis (BFR 8±6%, traditional 7±5%) also increased in both legs with training (p<0.05). The authors reported that: gross adaptations were greater in individuals with a greater percentage of Type II fibers, both the BFR and heavy load strength training increased gene expression, and hypertrophy from BFR was less than that of heavy load strength training in the proximal thigh (potentially due to the pressure from the cuff). The authors concluded that there were similar adaptations to training with both BFR and heavy load strength training in functional, physiological and cellular parameters in untrained women [119]

Lixandrão et al. (2015) investigated the effects of 12 weeks of BFR training at different occlusive pressures and exercise intensities on muscle mass and strength compared to traditional training in 35 sedentary males [120]. Quadriceps cross-sectional area and 1 RM for knee extension were assessed before and after the exercise intervention. Participants were then evenly distributed into one of five groups: 20% of 1RM with 40% occlusion, 20% 1RM with 80% occlusion, 40% 1RM with 40% occlusion, 40% 1RM with 80% occlusion, and 80% 1RM with no occlusion. Participants performed 12 weeks of biweekly unilateral knee extension training (2-3 sets of 15 reps for all except 80% 1 RM, which performed 10 reps per set), with 1 RM reassessed at 6 weeks to adjust training load. Blood flow restriction was achieved via a 92 mm wide pneumatic cuff. Differences were assessed between each BFR condition compared to the traditional resistance training. Quadriceps strength increased significantly in all but the 20% 1RM with 40% occlusion group (effect size for others 0.25-0.42). Increasing occlusive pressure only increased muscle cross section at lower intensities (effect size 0.74-2.54). Compared to traditional exercise, the interventions at 20% of 1RM had smaller increases in muscle size while those at 40% of 1RM were not significantly different from the traditional training. Quadriceps strength increased in all groups (10.30-21.60%, effect size 0.41-0.99), but was greatest with traditional training. The authors reported that the occlusion pressure only affected muscle hypertrophy at lower intensity levels, exercise intensity significantly influenced muscle cross sectional area in groups with similar occlusive pressures, only higher intensities were able to cause a similar hypertrophy response to traditional training and that neither pressure nor intensity were able to affect changes in strength with training and most likely had

smaller strength gains then the traditional training group. The authors concluded that exercise performed at lower exercise intensities (at least 20% 1 RM) could benefit from BFR, but this effect was not seen with higher intensities [120].

Vechin et al. (2015) compared the effects of 12 weeks of low intensity resistance training with BFR or tradition high intensity training on quadriceps muscle strength and size in 23 older individuals (59-71 years old) [59]. Leg strength was assessed using 1RM leg press, and quadriceps cross-sectional area was assessed using MRI. Blood flow restriction was achieved using an 18 cm wide cuff inflated to 50% of arterial pressure. Participants performed the leg press exercise two days per week for 12 weeks. The traditional strength training group completing 3 sets of 10 reps at 70% 1RM for the first 6 weeks and 3 sets of 10 reps at 80% 1 RM for the last 6 weeks. The BFR group completed 4 sets (30-15-15-15) at 20% 1 RM for the first 6 weeks and 30% of 1 RM for the last 6 weeks. Both groups had one minute of rest in between sets. Mixed model ANOVAs with Tukey post-hocs were used to assess differences in the dependent variables. There was a significant increase in leg press for the traditional resistance group (p<0.001, control ES 1.5, BFR ES 0.92), while the BFR group trended towards a significant increase (p=0.067, ES 0.59) and the control group remained unchanged (p=0.998). Quadriceps cross sectional area increased significantly with training for traditional training (p<0.001) and BFR training (p<0.001), but not control (p=0.395). Both the BFR and traditional training methods increased muscle strength and size compared to controls although traditional training methods resulted in a greater difference compared to BFR training (54% vs 17%) [59].

Yasuda et al. (2015b) investigated the effects of three weeks of detraining following six weeks of BFR training on muscular cross sectional area and strength in seventeen healthy males [58]. Bench press 1RM was used as the strength measurement and cross-sectional area was measured via MRI both before and after six weeks of training and then following three weeks of detraining. Participants were trained using either low intensity BFR (n=10, 30% 1RM, 30-15-15-15 reps with 30 seconds of rest) or traditional high intensity training (n=7, 75% 1RM, 3 sets of 10 reps with 3 min rest) three times a week for six weeks. Differences between groups and over time were assessed using repeated-measures ANOVAs (condition X time) with a Tukey post hoc test. Bench press 1RM was significantly higher following BFR and high intensity training and detraining (p<0.0001 for all). Cross-sectional area of the triceps brachii was also significantly increased following high intensity training and detraining (p<0.001 and p=0.0067 respectively), while the BFR group was only increased following training (p=0.0059). Pectoralis major cross-sectional area was significantly higher following training and detraining with traditional training (p<0.0001 and p=0.0059, respectively). Again, in the BFR group, cross sectional area was only increased following training (p=0.0001). Relative dynamic strength was significantly higher post training and detraining for the high intensity group (p<0.0001 for both), while the BFR group was only decreased following detraining (p=0.0064). The authors concluded that gains in strength derived from traditional high intensity training came from both neural adaptations and muscular hypertrophy while strength gains from BFR came primarily from muscular hypertrophy with training and from neural adaptations with detraining [58].

Yasuda et al. (2015b) investigated the effects of three weeks of detraining following six weeks of BFR training on muscular cross sectional area and strength in 17 healthy males [58]. Bench press 1RM was used as the strength measurement and cross-sectional area was measured via MRI both before and after six weeks of training and then following three weeks of detraining. Participants were trained using either low intensity BFR (n=10, 30% 1RM, 30-15-15-15 reps with 30 seconds of rest) or traditional high intensity training (n=7, 75% 1RM, 3 sets of 10 reps with 3 min rest) three times a week for six weeks. Differences between groups and over time were assessed using repeated-measures ANOVAs (condition X time) with a Tukey post hoc test. Bench press 1RM was significantly higher following BFR and high intensity training and detraining (p<0.0001 for all). Cross sectional area of the triceps brachii was also significantly increased following high intensity training and detraining (p<0.001 and p=0.0067 respectively), while the BFR group was only increased following training (p=0.0059). Pectoralis major cross-sectional area was significantly higher following training and detraining with traditional training (p<0.0001 and p=0.0059, respectively). Again, in the BFR group, cross sectional area was only increased following training (p=0.0001). Relative dynamic strength was significantly higher post training and detraining for the high intensity group (p<0.0001 for both), while the BFR group was only decreased following detraining (p=0.0064). The authors concluded that gains in strength derived from traditional high intensity training came from both neural adaptations and muscular hypertrophy while strength gains from BFR came primarily from muscular hypertrophy with training and from neural adaptations with detraining [58].

Shimizu et al. (2016) investigated the effects of 4 weeks of low-intensity BFR exercise on vascular endothelial function and peripheral blood circulation in forty elderly individuals [60]. Assessments were made before and after the first training session and after 4 weeks of training for 15 minutes three days per week. For each training session, BFR was applied using 10 cm wide cuffs on the thighs and 7 cm wide cuffs on the arms, inflated to the femoral and brachial systolic blood pressure, respectively. Training consisted of leg extensions, leg press, rowing, and chest press. Participants completed three sets of 20 reps at 20% of 1 RM with 30 seconds of rest between sets. During the first session, HR and ECG were monitored continuously and BP, blood lactate, epinephrine, and norepinephrine were measured prior to and after the session. Vascular endothelial function and peripheral blood circulation were measured prior to and after the training intervention. Two-by-two repeated measures ANOVAs were used to determine differences between and within groups. Acutely in the BFR group, heart rate, blood pressure, lactate and norepinephrine, vascular endothelial function and human growth hormone increased significantly following the initial training ($p<0.01$ for all) and were significantly higher than the non-BFR group ($p<0.01$ for all). Following four weeks of resistance training, estimated 1RM for leg extension, leg press and rowing in the BFR, but not non-BFR group ($p<0.01$). Measures of peripheral blood circulation and vascular endothelial function were significantly increased in the BFR group compared to controls following 4 weeks of resistance training. The authors concluded that BFR resistance training improved peripheral blood circulation and vascular endothelial function in healthy elderly people [60].

A meta-analysis by Loenneke et al. attempted to quantify which training variables

resulted in the greatest increases in muscle strength and hypertrophy when using BFR [87]. Of the studies selected for review, 11 were used in the paper and 19 were excluded. The mean effect sizes for the various training parameters for BFR training effecting muscular strength were: male (0.58), training status (untrained 1.38, trained 0.37), days per week (2-3 days 1.25, 4-5 days 0.53, 6-7 days 0.29), duration (≤4 weeks 0.27, 5-8 weeks 0.49, 9-10 weeks 1.38), type of training (isotonic 1.08, walking 0.42), intensity (15-30% 1.08), repetitions (60-70 1.37), rest (0 sec 0.50, 30 sec 1.22, 60 sec 0.25) and cuff pressure (140-220 mmHg 0.50, 160-240 mmHg 0.67). The mean effect sizes for the various training parameters for BFR training effecting muscular hypertrophy were: male (0.42), days per week (2-3 days 0.48, 4-5 days 0.27, 6-7 days 0.41), type of training (isotonic 1.08, walking 0.42), intensity (15-30% 1.08), type of assessment (isotonic 0.33, isometric 0.37), rest period (0 sec 0.37, 30 sec 0.44, 60 sec 0.35) and cuff pressure (140-220 mmHg 0.37, 160-240 mmHg 0.41). The authors concluded that BFR produced a much bigger response in combination with resistance exercise as opposed to walking and that training 2 or 3 days per week maximized the training adaptations. Additionally, neural adaptations to BFR training doesn't occur at the start of training, leading them to postulate that the initial increases in muscular strength may be due solely to muscular hypertrophy [87].

Low intensity resistance training with BFR has been shown to increase strength, hypertrophy, and endurance [60, 117-120]. Interestingly, these initial strength gains appear to come from hypertrophy, rather than neural adaptations as seen with traditional training methods [58]. However, this increase was less than that attained by traditional high intensity resistance training [59], although changes such as increased

peripheral blood circulation and vascular endothelial function were seen with both methods of training [60]. Of note is that there were similar adaptations to those seen with hypoxic training, such as translocation of GLUT-4 transporters to the muscle cell wall [61]. Low intensity resistance exercise with BFR appears to be an effective method for increasing strength and hypertrophy. No studies have yet investigated the changes in the oxidative stress following low intensity resistance training using BFR.

Practical Blood Flow Restriction

Blood Flow Restriction has also been studied using elastic knee wraps rather than pneumatic tourniquets to achieve venous occlusion. While it may not be able to achieve the precise occlusive pressures that the pneumatic systems are able too, they are much more readily available and practical in the sense that you can buy multiple sets of wraps for the price of a single pneumatic tourniquet. Making this method of BFR more suited to use by strength and conditioning coaches and other individuals with either need for use with many individuals or limited resources.

Loenneke et al. (2010) examined responses in RPE and pain to two sets of bilateral low intensity knee extension to failure with and without BFR from elastic knee wraps in 15 recreationally active individuals (9 men, 6 women). Participants performed the knee extension exercise at 30% 1RM and a 1 second concentric and 1 second eccentric motion to failure with 30 seconds of rest between sets. The same protocol was performed for both the control and BFR trials with the knee wraps put into place prior to the first set and removed following the second. Paired t tests were used to examine differences between groups. Repetitions to failure was significantly lower with BFR than control for the first (p=0.001, BFR 20.6±4.68 vs. control 27.86±6.12, ES 1.31) and

second set (p=0.001, BFR 4.26±1.66 vs. control 11.33±2.12, ES 3.66). Total work was also significantly reduced with BFR (p=0.001, 619.86±276.18 vs 962.25±344.14, ES 1.08). RPE was significantly higher in both sets (1st set: p=0.01, BFR 5.53±2.06 vs control 4.36±1.23; 2nd set: p=0.003, BFR 6.46±2.35 vs. control 5.10±0.43), but P! was only significantly different in the second set (1st set: p=0.15, BFR 5.00±2.58 vs control 4.20±1.97; 2nd set: p=0.009, BFR 6.43±2.63 vs. control 4.93±2.59). The authors concluded that pBFR decreased the time to failure compared to controls but did increase ratings of perceived exertion and pain, which may limit its use. They also suggested the potential of BFR for use in early rehabilitation [7].

Loenneke et al. (2012b) also investigated the use of elastic knee wraps in BFR walking and its effects on blood lactate. Nine male and female participants walked for five two-minute bouts of walking at 75 $m \cdot min^{-1}$ on a treadmill with 1 minute rest between bouts. Both the BFR and control trials were the same, except for the application of the 76 mm wide elastic knee wraps prior to the start of exercise that remained in place until the exercise was finished. Lactate concentration was assessed prior to exercise and immediately after the 1st, 3rd, and 5th set, and 3 minutes after the last set. Blood lactate levels were analyzed using 2x5 repeated measures ANOVA. While there was a significant change in blood lactate between conditions, this was most likely due to the response of one participant with BFR. The authors concluded that metabolic stress was not increased following pBFR walking, which may explain the lowered hormone response with BFR walking [121].

Yamanaka et al. investigated the effectiveness of four weeks of pBFR on hypertrophy and strength in NCAA D-IA football athletes [11]. Thirty two football

athletes with at least five years of resistance training experience, high levels of upper and lower body strength, and free of medical conditions. Resting BP, girth (upper and lower chest, upper and lower arm, height, body mass), and 1RM for bench press and squat were measured before and after the training intervention. Both the BFR and control groups performed the intervention as a supplemental program three days a week for 4 weeks. The intervention consisted of 1 set of 30 repetitions at 20% 1RM followed by 3 sets of 20% 1RM separated by 45 seconds of rest. Exercises were performed with the eccentric phase two times longer than the concentric phase. Elastic bands were 5 cm wide and were applied to restrict the brachial and femoral arteries and were overlapped 2 inches to restrict flow. Paired t tests were used to examine differences over time in each group. Independent t tests were used to compare differences between training effects. Changes in in BFR group 1RM were significantly greater for bench press (p<0.05, BFR 9.3±3.4 kg vs. control 4.1±4.2 kg, ES 1.2) and squat (p<0.05, BFR 14.0±3.8 kg vs. control 7.2±5.3 kg, ES 1.3). Changes in left upper arm girth and lower chest girth were also significantly greater in the BFR group (p<0.05 for both). It was concluded that low-intensity exercise combined with BFR resulted in greater muscle hypertrophy and strength (bench press 7.0% vs 3.2%, squat 8.0% vs 4.9%) [11].

Wilson et al. examined the effects of moderate pBFR on metabolic stress, muscle swelling, skeletal muscle activation, and indices of muscle damage following an acute bout of low intensity exercise in 12 college aged male participants with at least one year of resistance training. This study also served to validate a practical method of BFR using elastic knee wraps. Blood flow in the femoral artery and vein was assessed

using Doppler ultrasound. During the first visit, participants had their blood flow measured with BFR as well as performed 1RM testing. Prior to measurement, the participants had their thighs wrapped with 7.6 cm wide elastic knee wraps after being introduced to the authors perceived pressure scale: 0 out of 10 pressure meant no pressure; a 7 out of 10 was described as moderate pressure with no pain; and a 10 out of 10 pressure was described as intense pressure with pain. The wraps were placed around the femur distal to the inguinal fold and the participants were asked about the perceived pressure after each pass of the elastic wrap. Three different wrapping intensities were used: 0 out of 10 (control), 7 out of 10 (moderate), and 10 out of 10 (tight). Participant's leg press 1RM was then measured. There was no BFR with the control condition, the femoral vein, but not the femoral artery, was occluded at the 7 out of 10 pressure level, and both the femoral artery and vein were occluded in 67% of participants with the 10 out of 10 pressure level. The moderate pressure resulted in greater indicators of metabolic stress ($p<0.05$, ES = 0.2, blood lactate 6.2±2.8 mmol/L vs. 4.7±1.8 mmol/L after exercise), muscle swelling ($p<0.05$, increased immediately post exercise and through 5 minutes post-exercise) and muscle activation ($p<0.05$, ES=0.2) than a volume matched control. The authors suggest that while the venous occlusion did play some role in increased muscle thickness, the continued muscle swelling 5 minutes after the removal of the wraps indicates that there was a fluid shift into the muscle cells at some point and the increased muscle activation is due to the recruitment of type II muscle fibers through the stimulation of group III and IV afferent nerve fibers. It was concluded that moderate BFR can acutely increase metabolic stress, skeletal

muscle swelling and muscle activation using elastic knee wraps to occlude the veins, but not arteries (pressure 7 out of 10) [10].

Lowery et al. compared the effects of a pBFR program on muscle hypertrophy compared the traditional high intensity programs. Twenty college aged males with at least one year of resistance training were included in this study and resistance load was assessed using a predicted 1RM from a 10RM. This randomized, four-week crossover protocol (eight weeks total) also controlled for overall total volume between arms of the study. During the high intensity program, participants performed half of the reps at twice the resistance of that performed with BFR. Muscle thickness of the dominant arm was tested using ultrasound at baseline, 4 weeks, and at 8 weeks. Repeated measures ANOVAs with Tukey post hoc test were used to assess differences between and within groups. Both groups increased significantly from baseline to 4 weeks ($p<0.01$) and from 4 weeks to 8 weeks ($p<0.01$), but there were no significant differences between the BFR and high intensity training protocols. Therefore, pBFR training resulted in similar hypertrophy gains as volume matched traditional high intensity training [8].

Luebbers et al. investigated the effects of 7-weeks of pBFR training combined with a traditional weight training program on muscle strength and size in 62 DII collegiate football players. Upper and lower body girths, 1RM Bench and 1RM squat was assessed before and after the intervention. Participants performed either a traditional high intensity training program (H), the high intensity protocol supplemented with a 20% 1RM protocol (H/S), and the high intensity protocol supplemented with a 20% 1RM protocol performed with BFR (H/S/R), and a modified training program (M) that didn't include high intensity bench press and squat and the supplemental 20% 1RM

protocol performed with BFR (M/S/R). The supplemental protocol consisted of 4 total sets (1 set of 30 reps followed by 3 sets of 20 reps) at 20% 1RM with 45 seconds rest between sets. The eccentric and concentric phases were both performed for 1.5 seconds. A 4x2 MANCOVA was used for analysis with training group set as the between factor, time as the within factor and body mass as the covariate. One-way 1x4 ANOVAs and LSD post hoc tests were used to further investigate any significant main effects from the MANCOVA. The H/S/R group had a significantly larger increase in 1RM squat (24.87 kg) than the other three training groups (M/S/R 5.97 kg, $p<0.001$; H/S 14.13 kg, $p=0.025$, H 13.64 kg, $p=0.009$). There was a significant increase in 1RM bench press over time ($p<0.001$), but the 1x4 ANOVA failed to reveal differences between groups ($p>0.180$). There was a significant main effect across groups for arm and leg circumference (arm $p=0.046$, thigh $p<0.001$), but not chest ($p=0.739$). However, the 1x4 ANOVA revealed no differences between groups for any girth measurement (arm $p=0.424$, thigh $p=0.958$, chest $p=0.381$). The authors concluded that: the H/S/R group significantly larger increases in 1RM squat, there was a significant increase in 1RM bench press across groups, the supplemental lifting program, with or without BFR, made no difference on the effect size of 1RM gains; and similarly, the thigh and arm circumferences had a significant main effect but no differences between groups. This study was similar to one performed by Yamanaka et al., with the addition of the M/S/R and H groups for further comparison as well as the use of 7.6 cm wide elastic knee wraps with 7.6 cm of overlap instead of 5 cm wide elastic cuffs tightened to achieve 5.1 cm of overlap. The authors conclude that pBFR training can increase 1RM squat performance when used in conjunction with off-season high intensity training with

collegiate football athletes.[9] Additionally, the use of inexpensive elastic knee wraps to induce BFR make BFR training a more viable option for larger groups or clinics with less resources.

Practical Blood Flow Restriction using elastic knee wraps appears to be a practical method of achieving the beneficial results of BFR training [7-11] without the use of expensive equipment and can be utilized in a variety of settings, such as supplemental training for athletes or those who are unable to perform traditional high intensity resistance training. This version of BFR is much more likely to be utilized in a variety of settings due to its low cost and ease of implementation in a group setting. Additionally, benefits are still seen when performing pBFR training.

Oxidative Stress

Oxidative stress is defined as an imbalance between antioxidants and reactive oxidant species (ROS). This occurs when either the rate of ROS production overwhelms antioxidant production, antioxidant production decreases below the level needed to keep the ROS from starting a chain reaction, or both [122]. While oxidative stress is commonly referred to as a bad condition by the media, with foods rich in antioxidants or antioxidant supplements often touted as being beneficial, the ingestion of too much antioxidants will result in a decrease in the body's natural production of antioxidants in response to exercise [21]. Additionally, oxidative stress due to exercise is actually the mechanism that triggers many physiological pathways that are beneficial, such as AMPK [44, 45].

Bloomer and Fisher-Wellman investigated the effects of training level, gender, and diet on resting levels of oxidative stress in DNA, proteins, and lipids. One hundred

and thirty-one individuals consisted of 89 men (74 trained) and 42 women (22 trained). Blood was drawn from participants who fasted for 8 hours and refrained from strenuous exercise for 48 hours prior to blood collection. Additionally, participants recorded all food and drink consumed for the five days prior to the blood draw. Oxidative stress on DNA (8-OHdG), protein (PC), and lipids (MDA) were measured and a 2x2 ANOVA was run. Additionally, Stepwise regression and correlations were run to determine relationships between gender, training status, antioxidant consumption, and oxidative stress biomarkers. There were no differences between trained men and women or between untrained men and women in PC levels; however, when combined, PC levels were significantly lower in trained vs. untrained individuals ($p<0.05$). There were no significant differences between groups for 8-OHdG. Mean MDA levels were significantly lower in: trained men vs trained women, overall trained vs untrained, and women vs. men ($p<0.05$ for all). Additionally, vitamin C intake ($R^2=0.062$) and both Vitamin C and sex ($R^2=0.138$) were significant predictors in the regression model. Oxidative stress biomarkers were significantly lower in trained vs untrained participants for PC and MDA, but there were no significant differences in 8-OHdG for both gender and training status. The authors concluded that MDA was lower in women compared with men, both PC and MDA were lower in trained vs untrained individuals, except for MDA in trained men, dietary intake was not correlated with biomarkers of oxidative stress [15].

Tanskanen et al. investigated the relationship between overtraining syndrome and oxidative stress in seven over-trained and 10 control athletes [20]. Measurements were collected at baseline and after a six-month recovery period. Venous blood was

collected before and immediately after a VO_{2MAX} test at these time points and analyzed for protein carbonyls, malondialdehyde, and oxygen radical absorbance capacity. Differences were assessed between groups using Friedman and Wilcoxon pairwise tests. Protein carbonyls were higher at rest in the over-trained participants at baseline (p=0.003, effect size 0.40). Exercise to exhaustion increased protein carbonyls in the control group (p=0.017, effect size 0.33), but not the over-trained group (p=0.772, effect size 0.00). Six months of recovery did not change protein carbonyls in either group (control p=0.123, effect size 0.16; over-trained p=0.370, effect size 0.06). Malondialdehyde increased with exercise in controls at baseline (p=0.001, effect size 0.56) and at six months (p=0.0001, effect size 0.64), but there was no change in the over-trained group at baseline or six months (p>0.05 for both). Oxygen radical absorbance capacity increased with exercise in the controls at baseline (p=0.008, effect size 0.40) and after six months (p=0.003, effect size 0.47) but not with over-trained group (baseline p=0.323, effect size 0.05; 6 months p=0.928, effect size 0.00). The authors concluded that antioxidant protection against oxidative stress from exercise was impaired in resting over-trained athletes [20].

Hadžović-Džuvo et al. investigated the oxidative stress status of 39 elite athletes from three different sport backgrounds (12 wrestlers, 14 soccer players, and 13 basketball players) [17]. Blood samples were taken and assessed for advanced oxidation protein products, malondialdehyde and total antioxidative capacity. Differences between groups were assessed using one-way ANOVAs with a Tukey post-hoc test. Mean advanced oxidation protein products and total antioxidative capacity was not significantly different between groups (p=0.424 and 0.133 respectively).

Malondialdehyde was significantly higher in basketball athletes compared to soccer players (p=0.003). Wrestlers were not significantly different from both basketball and soccer athletes (p>0.05). No measures of oxidative stress or antioxidants were significantly correlated with anthropometrics, between sports, or ingestion of antioxidant supplements. The authors concluded that the type of sport played had no effect on markers of oxidative stress [17].

Kliszczewicz et al. investigated oxidative stress in the blood following both a CrossFit™ workout and control in ten male participants [25]. Prior to the start of the study, VO_{2MAX} was assessed using the Bruce protocol and body fat was assessed using Dual Energy X-Ray Absorptiometry (DXA). The CrossFit™ workout consisted of as many rounds as possible of 5 pull-ups, 10 push-ups, and 15 air-squats in 20 minutes, using body mass as the resistance. The control exercise was a 20 minute run on a treadmill at 90% of maximum heart rate. Blood was drawn before, immediately after, and 1 and 2 hours post-exercise and analyzed for markers of oxidative damage (lipid hydroperoxides and protein carbonyls) and antioxidant capacity [Ferric-reducing antioxidant power (FRAP) and Trolox-equivalent antioxidant capacity (TEAC)]. Lipid oxidative damage increased significantly at one hour (CrossFit™ 170±36.9% of Pre, treadmill 146±36.9% of Pre, p<0.001 for both) and two hours (CrossFit™ 351±107.3% of Pre, treadmill 205±59.1% of Pre, p<0.001 for both) post exercise for both conditions but did not differ between conditions (p=0.623). Protein carbonyls decreased over time regardless of condition (p=0.001). All post-exercise time points had increased FRAP for both conditions (p<0.001 for all), however, TEAC decreased at all post-exercise time points in both conditions (p=0.001 for all). The authors concluded that the intensity,

rather that the type, of the exercise bout was the main reason for increases in lipid hydroperoxides and that markers of oxidative damage and antioxidants responded similarly in both CrossFit™ and high-intensity treadmill running [25].

Oxidative Stress and Aerobic Exercise

Oxidative stress is often measured with aerobic exercise since approximately one to five percent of oxygen molecules consumed during respiration are improperly reduced and converted into ROS [123]. Thus, as the aerobic energy pathways start to increase in activity the rate of ROS production increases in order to provide ATP. Thus the measurement of oxidative stress with exercise is of clinical importance.

Elosua et al. assessed the effects of 16-week individualized aerobic exercise program on antioxidant activity and LDL oxidation in 17 sedentary participants [19]. A graded exercise test was performed at the start and end of the exercise intervention to assess VO_{2MAX}, ventilatory threshold and aerobic power output. Participants then performed 30 minutes of physical activity at the level of their maximal aerobic power output. Blood samples were collected at rest, immediately after, 30 minutes after, and 1, 2, and 24 hours post exercise and assessed for superoxide dismutase activity in erythrocytes, oxidized glutathione, reduced glutathione, and LDL oxidation. Paired t-tests and MANOVAs were used to assess differences. There were significant increases in VO_{2MAX}, ventilatory threshold, and aerobic power output, but no differences in blood markers of triglycerides. Glutathione reductase activity increased 15 to 23% with training ($p<0.05$), but there was no significant difference in erythrocyte superoxide dismutase activity with aerobic training while LDL cholesterol became more resistance to oxidation (increase in lag time, decrease in maximal oxidation rate). Prior to training

there was a significant increase in erythrocyte superoxide dismutase 24 hours after exercise that was not present after training. Erythrocyte superoxide dismutase activity was significantly higher after training at 30 minutes and 1, 2, and 24 hours post-exercise. The authors concluded that regular physical activity increased antioxidant activity and LDL resistance to oxidation as well as decreasing the oxidized LDL concentration but that the time response pattern to acute aerobic exercise remains similar before and after 16 weeks of training, although the levels did change [19].

Quindry et al. investigated whether exercise induced neutrophilia causes oxidative stress in the blood and if total energy expenditure or exercise intensity was the main agent of change in nine male participants [27]. A Broeder treadmill protocol was utilized to measure VO_{2MAX} and a lactate threshold test (determination of point at which blood lactate rose more than one millimole above resting levels) was also administered during a separate testing session. Three more submaximal exercise protocols were then conducted in three separate visits (10% above lactate threshold for 45 min, 10% below lacate threshold for 45 minutes, and 10% below lactate threshold until energy was the same as the 10% above lactate threshold). Blood was drawn before, immediately after, and 1 and 2 hours after exercise) and corrected for plasma volume shift prior to analysis for white blood cell counts, superoxide anion, malondialdehyde, and ascorbic acid. Repeated measures ANOVA were used to assess difference over time and between conditions. Only conditions performed above lactate threshold increased neutrophil count significantly above baseline (VO_{2MAX} 73%, 10% > lactate threshold 43%). Superoxide anion was also increased in these conditions (VO_{2MAX} - immediately after and 2 hours post exercise; 10% > lactate threshold 2 hours post

exercise). Blood lactate had a positive correlation with neutrophil counts (r=0.97, *p*=0.009), while total energy expenditure did not have any significant correlation with neutrophil count (r=0.59, *p*=0.229). The authors concluded that significant blood oxidative stress was observed after only maximal exercise, along with increased neutrophilia and neutrophil-generated superoxide anion levels [27].

Dayan et al. investigated the effects of a maximal graded exercise test on susceptibility of serum lipids to peroxidation in 30 males [28]. Participants performed a VO_{2MAX} test on a treadmill ergometer and venous blood was drawn before and after the test. Blood lipids (total cholesterol, triglycerides, LDL, and LDL), Vitamin E, and the susceptibility of serum lipids to oxidation were evaluated for both samples. Differences between pre and post blood samples were calculated and partial correlations were determined between variables. There was no significant correlation between variables of VO_{2MAX} and oxidation of serum lipids. The authors concluded that short-term graded exercise (8 to12 minutes) had no effect on the kinetics of serum lipid peroxidation [28].

Ristow et al. investigated the effects of antioxidant supplementation on the occurrence of oxidative stress with physical exercise in 40 participants (20 trained, 20 untrained) [21]. Half of each group (10 trained, 10 untrained) received antioxidant supplementation of vitamins C and E during a 4-week aerobic exercise intervention. Insulin sensitivity and measures of antioxidant defense were measured before and after the intervention. Only the members of the non-antioxidant group (both trained and untrained) had increased levels of insulin sensitivity and antioxidants ($p<0.001$ for both trained and untrained). Therefore, the authors concluded that antioxidant

supplementation blocked the beneficial effects of physical exercise by limiting the transient increase in oxidative stress that normally occurs with exercise [21].

Cubrilo et al. investigated the differences between athletes from rowing (n=13), cycling (n=10), and taekwondo (n=20) in the oxidative stress response to a sport specific VO_{2MAX} test [124]. Participants had their VO_{2MAX} and anaerobic threshold measured via graded exercise testing conducted on a rowing ergometer (rowers), cycle ergometer (cyclists), and treadmill (taekwondo). Blood samples were collected prior to, immediately after, and 4 and 10 minutes after the test and assessed for malondialdehyde, superoxide anion, nitrate, and blood lactate concentrations. Two-way ANOVAs with a Tukey post-hoc were used to assess differences in responses. Blood lactate response did not differ between groups. Nitrite concentrations at rest were significantly different between groups ($p<0.001$), with taekwondo having the lowest levels, followed by cyclists and then rowers; however, these levels did not change with exercise. Malondialdehyde was also different at rest between groups ($p<0.001$), with rowing having the lowest concentration and cycling having the highest concentration. These levels did not change with exercise. The authors concluded that training was able to provoke upregulation of resting levels of nitrates and products of lipid peroxide but maximal exercise caused no changes in these parameters at maximal load or during 10 minutes of recovery following exercise [124].

Djordjevic et al. compared the effects of maximal aerobic exercise on pro- and antioxidants in athletes and non-athletes (58 athletes, 37 non-athletes) and examined the correlations between anthropometrics and pro- and antioxidants levels before and after exercise [18]. Anthropometric measurements (height, body mass, body fat

percentage) were recorded prior to exercise. Participants performed a maximal graded exercise test on a cycle ergometer and their VO_{2MAX} was recorded. Blood was drawn before and immediately following exercise and assess for superoxide anion, hydrogen peroxide, nitric oxide, malondialdehyde, catalase, superoxide dismutase, and reduced glutathione. Two-way repeated measures ANOVA were used to assess differences between groups and with exercise. Pearson's coefficients of correlation were also calculated between variables. Athletes had significantly higher resting levels of nitric oxide, superoxide dismutase, catalase, and reduced glutathione and lower levels of malondialdehyde compared to non-athletes. With exercise, non-athletes experienced a significant rise in superoxide anion, hydrogen peroxide and a significant decrease in malondialdehyde while athletes experience a significant decrease in nitric oxide and catalase. The only significant difference between athletes and non-athletes was the significantly lower levels of superoxide anion. Percent body fat was positively correlated with hydrogen peroxide levels before and after exercise (r=0.335 and 0.309 respectively, $p<0.05$ for both) and with malondialdehyde after exercise (r-0.3589, $p<0.01$). Following exercise, percent muscle mass had a negative correlation with hydrogen peroxide (-0.347, $p<0.01$) and a positive correlation with malondialdehyde (r=0.323, $p<0.05$). Maximal oxygen uptake was negatively correlated with: hydrogen peroxide prior to exercise (r=-0.366, $p<0.01$), malondialdehyde both before (r=-0.328, $p<0.05$) and after exercise (r=-0.279, $p<0.05$), and nitric oxide following exercise (r=-0.295, $p<0.05$). Maximal oxygen uptake was also positively correlated with catalase prior to exercise (r=0.326, $p<0.05$). The authors concluded that athletes were under lower risk of oxidative stress both at rest and following exercise and suggested that

improved physical fitness may improve antioxidant defense and preserve an individual's health [18].

Gwozdzinski et al. investigated the change in erythrocyte and plasma oxidative status following an acute bout of exercise in 11 untrained males [29]. Participants performed a graded exercise test to exhaustion on a cycle ergometer. Maximal oxygen consumption was then estimated using time to exhaustion. Venous blood samples were collected at rest, immediately post exercise, and one hour post exercise and analyzed for blood lactate concentration, hemoglobin, and hematocrit. Erythrocytes were separated and washed and analyzed for hemoglobin using electron paramagnetic resonance. Erythrocyte membrane peroxides did not differ following exercise although acetylcholinesterase activity (a marker inhibited by oxidative stress) was insignificantly decreased immediately after and one hour after exercise ($p>0.05$). Hemoglobin was found to have increased conformational changes immediately after exercise as well as a decreased concentration of ascorbic acid inside erythrocytes. The authors concluded that a single bout of aerobic exercise can lead to increased antioxidant activity in the blood in young untrained males [29].

Ugras investigated the effects of high-intensity interval training on oxidative stress in elite Muay Thai athletes (15 male, 6 female) [62]. Blood samples were collected prior to training camp, after training camp and prior to competition, and at the end of competition. Erythrocytes were then analyzed for superoxide dismutase, GPX, catalase and malondialdehyde were then measured. Differences in oxidative stress parameters were measured using Wilcoxon Signed Ranks tests. The training protocol consisted of two 1.5-hour practices per day for 10 days. Erythrocyte levels of

malondialdehyde increased post-training and post-competition (p<0.05 for both), while levels of catalase only decreased post-competition (p<0.05). There were no significant changes in superoxide dismutase or GPX, although there was reduction noted in superoxide dismutase. The authors concluded that high-intensity interval training and competition can change the oxidative status of elite Muay Thai athletes [62].

Ahn and Kim investigated if changes in MDA or superoxide dismutase (SOD) as well as other physiological responses were different between eight obese (BF% ≥25%) and eight lean (BF%<20%) individuals after 1 hour of submaximal exercise in normal or cold environments [24]. Participants performed 60 minutes of submaximal cycling at 60% of VO_{2MAX} in both normal (21±1°C, 65±2% relative humidity) and cold (4±1°C, 30±1% relative humidity) conditions. Heart rate, lactate concentrations, and core temperature were measured before exercise, at 10-minute intervals during exercise, and at 5, 10, 15, and 30 minutes post exercise. Blood samples were collected at rest, at 10 minute intervals during exercise, and 15 and 30 minutes post exercise and analyzed for MDA, and SOD. Repeated measures ANOVAs were used to calculate differences between groups and conditions. Heart rate increase was significantly lower in the cold condition for both groups (p<0.05). Core temperature had a significant time by condition by group interaction (p<0.05). Core temperature was higher in the obese group in normal conditions. Levels of MDA changed in both groups over time (p<0.05), but was not significantly different between conditions or groups. Levels of SOD were significantly different between conditions (p<0.05) and groups (p<0.05). The increase in SOD activity was significantly greater in the cold condition (p<0.05), and remained elevated for longer in the obese group. The authors concluded that the cold conditions

challenged antioxidant defense mechanisms in the obese group, while lipid peroxidation remained similar to that during normal conditions [24].

Parker et al. investigated the relationship between exercise intensity and oxidative stress and total antioxidant capacity in 14 healthy young males [30]. Participants completed an incremental cycle protocol to determine VO_{2MAX} and maximum work load. One week after the $VO2_{MAX}$ participants performed an intermittent cycle protocol which consisted of five minutes of steady state exercise performed at 80 RPM at 40, 55, 70, 85, and 100% of VO_{2MAX} with 12 minutes of seated rest between stages. Heart rate and RPE were recorded every minute and capillary blood samples were collected at rest and at the end of each stage for analysis of blood lactate concentration, oxidative stress and total antioxidant capacity, systemic oxidative stress and biological antioxidant potential was also assessed. Increased exercise intensity had no significant effect ($p>0.05$) on systemic oxidative stress compared to resting values while biological antioxidant potential was significantly greater at 70, 85, and 100% of VO_{2MAX} ($p=0.009$, 0.005, and <0.001 respectively) compared to resting levels. As expected, HR, RPE, blood lactate concentration, and VO_{2MAX} increased with increased exercise intensity. The authors concluded that high-intensity short duration exercise significantly increases antioxidant levels compared to rest and low-intensity exercise and did not increase oxidative stress. Total antioxidant capacity increase with exercise may be indicative of a transient increase in production of reactive oxygen species, which may lead to health benefits and protect against chronic diseases related to oxidative stress [30].

Wiecek et al. (2015a) investigated if maximal-intensity exercise induced the same changes in total oxidative stress, antioxidative capacity, and uric acid between males and females (total n=20) [16]. Participants performed a treadmill VO_{2MAX} test and blood samples were collected 5 minutes before and 3 minutes after the test. Blood was then analyzed for lactate concentration, acid-base balance, hydrogen ion concentration, bicarbonate ion concentration, base excess/deficit, and anion gap. Blood was also collected 5 minutes prior to, and 10 minutes and 24 hours after exercise testing for analysis of total oxidative status, total antioxidative capacity, and uric acid concentration. Differences between sexes were assessed using repeated measures ANOVA (time vs sex). Maximal oxygen uptake was approximately 25% lower in women compared to men (44.48±1.21 vs 59.16±1.55 $mL \cdot kg^{-1} \cdot min^{-1}$, $p<0.01$). Blood lactate and anion gap concentrations were significantly higher 3 minutes post exercise in men compared to women ($p<0.05$). All acid-base variables changed significantly with maximal exercise testing ($p<0.01$). Women had higher levels of total oxidative status, oxidative stress indicator than men throughout testing ($p<0.05$). Exercise testing increased total oxidative status in both sexes; but the changes was significantly greater in men (men 43%, women 6.5%, $p<0.01$). Total oxidative status decreased significantly from post-test values at 24 hours post-exercise in men while levels of total antioxidant capacity increased significantly in women ($p<0.05$ for both). Men had a significant correlation between increase in total oxidative status with exercise and absolute VO_{2MAX} (r=0.80), lean body mass ratio-scaled VO_{2MAX} (r=0.72), and blood lactate concentration increase (r=0.65). The authors concluded that maximal exercise testing caused

significantly different changes in the balance between oxidants and antioxidants between men and women [16].

Oxidative Stress and Resistance Exercise

Alessio et al. compared biomarkers of oxidative stress after exhaustive aerobic and isometric exercise in twelve participants [41]. All participants performed a VO_{2MAX} test on a treadmill using standard open circuit spirometry. One week later, they had their dominant handgrip strength tested. Following a rest, participants held 50% of handgrip strength for 45 seconds, followed by 45 seconds of rest, until they had exercised for the same amount of time as with the treadmill test. Blood samples were collected prior to, immediately after, and 1-hour post-exercise and analyzed for lipid hydroperoxides (LH), Malondialdehyde (MDA), protein carbonyls, lactate concentration, and oxygen radical absorbance capacity (ORAC). Both HR and MAP increased more with the VO_{2MAX} test compared to isometric handgrip ($p<0.05$), blood lactate followed a similar path and increased 479% following the VO_{2MAX} and 221% following the isometric handgrip ($p=0.004$). There were no significant changes in MDA at any time point. Lipid hydroperoxides did increase significantly after isometric handgrip (36.2%, $p=0.001$). Protein carbonyls increased 67% immediately post VO_{2MAX} compared with a 12% increase following isometric handgrip ($p=0.01$). Markers of antioxidants (ORAC) increased more following VO_{2MAX} (25%, $p=0.01$) than isometric handgrip (9%, $p>001$), but these values were not significantly different at 1-hour post-exercise. The authors concluded that lipids underwent peroxidation more during exhaustive isometric exercise while proteins were more likely to by oxidized during aerobic exercise. Therefore, different types of exercise cause different types of oxidative stress [41].

Groussard et al. directly measured lipid radical production following the WAnT using electron spin resonance spectroscopy in eight healthy males [33]. Participants had their VO_{2MAX} estimated via an Astrand submaximal cycle program prior to the study. A WAnT was then performed on a separate day. Blood samples were collected prior to and 5, 10, 20, and 40 minutes post WAnT and analyzed for thiobarbituric acid reactive substances, erythrocyte glutathione, glutathione peroxidase, and superoxide dismutase as well as electron spin resonance and hematocrit. Electron spin resonance was performed pre- and 5, 10 and 40 minutes post-WAnT. Differences between time-points were assessed using one-way ANOVAs. Electron spin resonance revealed a progressive increase in the kinetics of lipid radical production (up to 2.7 times resting levels) that became significantly difference from rest at 20 minutes post-WAnT (p<0.05). However, there was no similar pattern in plasma thiobarbituric acid reactive substances concentrations, which decreased significantly at 20 and 40 minutes post-WAnT (p<0.05). Superoxide dismutase activity in erythrocytes decreased significantly immediately post-WAnT (p<0.05). Glutathione peroxidase did not significantly change at any time-point while glutathione had a nonsignificant increase at 5 minutes post-WAnT. A preliminary study verified that there was no significant increase in lipid radical levels with submaximal aerobic exercise similar to the warm-up for the WAnT, thus changes in lipid radical levels were due solely to the supramaximal exercise performed during the WAnT itself. The authors concluded that short-term supramaximal anaerobic exercise such as the WAnT induces oxidative stress, as indicated by the increase in lipid radical levels and promotes malondialdehyde removal, suggesting that the

thiobarbituric acid reactive substances assay is on a suitable marker of oxidative stress for this type of exercise [33].

Baker et al. compared cycle ergometry performance using measures based off of total body mass and fat free mass and compared its effects on levels of malondialdehyde, lipid hyperperoxide, creatine kinase, myoglobin, and blood lactate in eighteen physically active males [31]. Participants performed a force-velocity cycling test 1 week prior to the study, which was used for a WAnT using resistance determined using total body mass or fat free mass. Peak and mean power were recorded and blood samples were recorded prior to and immediately following exercise, as well as 24 hours post-exercise. Samples were then assessed for malondialdehyde, lipid hyperperoxide, cardiac troponin and myoglobin, retinol and alpha-tocopherol. One-way repeated measures ANOVAs with Tukey post-hoc were used to assess differences. Lipid hyperperoxide, malondialdehyde, and myoglobin concentrations increased immediately post-exercise and decreased 24 hours later using resistance calculated with total body mass ($p<0.05$). Creatine kinase increased with both protocols immediately after exercise and decreased at 24 hours ($p<0.05$). Cardiac troponin, retinol, and alpha-tocopherol decreased with both protocols immediately after exercise and increased at 24 hours ($p<0.05$). Lactate concentrations increased after exercise and decreased at 24 hours for both protocols ($p<0.05$). The WAnT using resistance determined from fat free mass resulted in significantly less oxidative stress compared to that using total body mass. Additionally, the authors concluded that increased oxygen

consumption is not the only mechanism associated with damage due to oxidative stress [31].

Goldfarb et al. (2005) investigated the effects of antioxidant therapy on markers of oxidative stress and antioxidants in 18 untrained females after eccentric resistance exercise [22]. Participants were randomly assigned to either an antioxidant supplement (n=9) or a control group (n=9). The antioxidant supplementation consisted of 400 IU of vitamin E, 1 g of vitamin C, and 90 µg of selenium, taken 3 times a day for 2 weeks prior to exercise sessions while those in the placebo group took identical placebo capsules over the same period. During the exercise session participants performed four sets of 12 repetitions of concentric-eccentric elbow curls using their nondominant arm at a velocity of 20° per second with one minute of rest in between sets. Blood samples were collected prior to exercise and 0, 2, 6, 24, and 48 hours after exercise. Blood was then assessed for plasma proteins, protein carbonyls (oxidative damage to proteins), malondialdehyde (oxidative damage to lipids), and total body whole glutathione, and oxidized glutathione (antioxidants). A 2x6 repeated measures ANOVA was used to analyze the data. There was no group by time effect for protein carbonyls, but levels were significantly higher 24 and 48 hours post exercise compared to pre-exercise levels in both groups and the increase of PC in the control group was ~200%, when that of supplemental group was only ~50%. Malondialdehyde was significantly higher at 48 hours post exercise compared to pre-exercise; additionally, levels in the control group were significantly lower in the control group compared to the supplemental group at 48 hours. There were no significant differences between groups for total whole-body glutathione, oxidized glutathione, glutathione, or the ratio between the two. However,

there was a significant decrease in total whole-body glutathione, and glutathione immediately after and 2 hours after exercise compared to pre-exercise, while oxidized glutathione was increased during this time. The authors concluded that eccentric exercise increased biomarkers of oxidative stress, especially for protein oxidation, and that antioxidant supplementation can attenuate that response in untrained women [22].

Bloomer et al. (2006) investigated the responses in oxidative stress and markers of skeletal muscle injury to work matched sprints and resistance exercises in twelve anaerobically trained males [125]. Participant anthropometrics and 1RM squat max were determined 1 week prior to testing. Isometric leg strength was also collected. Sprint cycle ergometry was evaluated first to match the workload performed during the squatting exercise; participants performed six 10 second maximal sprints with 3 minutes of recovery between sets. Total work was then calculated and used to determine the number of repetitions needed for the barbell squat exercise. Participants performed squats at 70% of 1RM to failure with 3 minutes rest in between sets until they had matched the work performed during the cycle sprint test. Venous blood samples were collected before each session and 10 and 30 minutes after the completion of each exercise session as well as 24 and 48 hours post exercise. Only blood samples collected the day of testing were analyzed for whole blood lactate, while all samples were tested for hematocrit and hemoglobin, creatine kinase, protein carbonyls, and malondialdehyde. Additionally, measures of muscle soreness and force production were collected at the same time-points as blood collection. Muscle biopsies were collected 2 weeks prior to and 24 hours after exercises on eight participants and analyzed for fiber type and desmin. Variables were analyzed using repeated measures

ANOVAs (exercise session X time). Blood lactate was higher for both post-exercise time points following sprint cycling compared to squats (10 min: 10.3±0.51 vs 7.9±0.54 mmol·L^{-1}, 30 min: 6.0±0.56 vs 3.3±0.24 mmol·L^{-1}, p=0.002). While there was no interaction effect (p>0.05), there was an effect for both exercise type (p=0.003) and time (p=0.0003) for muscle soreness, with increased values with squatting, and peaking immediately post-exercise. There were no changes in isometric force production or rate of force development (p>0.05). Creatine kinase had a significant time effect, peaking after 48 hours for both exercise sessions (p=0.048). Muscles were 45.9±1.3% Type I fibers and 54.1±1.3% Type II fibers. Desmin did not differ with either type of exercise session. Differences in protein carbonyls post exercise did not differ between sessions (p=0.447, effect size 0.308). Malondialdehyde trended towards a significant decrease following exercise (p=0.0672), but no difference between sessions (effect size 0.149). Neither exercise session was sufficient to cause significant changes in markers of oxidative stress in anaerobically trained males, leading the authors to conclude that strenuous bouts of squats of sprint cycling are not associated with oxidative stress and markers of skeletal muscle injury [125].

Dixon et al. investigated the responses of serum malondialdehyde to acute resistance training and if there was a different response between training experience [126]. Participants were college aged men with either 1 year of continuous resistance training experience (n=12) or no regular aerobic or resistance training within the past year (n=12). Prior to the study, participants underwent a treadmill VO_{2MAX} test using a Bruce protocol and 1RM testing for chest press, lat pull-down, leg extension. Leg curl, bicep curl, triceps extension, front row and inverted leg press. The protocol consisted of

three circuits of the eight exercises performed at 73.5% of 1RM (10RM) with 2 minutes of rest between stations during the first circuit, 1.5 minutes during the second circuit, and 1 minute during the third circuit. Blood samples were collected at rest, 5 minutes post-exercise and 6, 24, and 48 hours post exercise. Samples were assessed for hematocrit, blood lactate, malondialdehyde, and creatine kinase. Repeated measures ANOVA (group X time) were used to analyze oxidative stress and creatine kinase. As expected, the trained group lifted significantly more weight throughout the circuit ($p<0.05$). Blood lactate concentrations increased similarly between groups ($p<0.05$ over time, $p>0.05$ between groups). Malondialdehyde levels did not change between or within groups ($p>0.05$ for both). Creatine kinase was significantly increased at all time points following resistance training. The authors concluded that since moderate-intensity whole-body resistance training had no effect on malondialdehyde in both trained and untrained males, the intensity used by many recreational lifters and athletes is insufficient to cause muscle damage due to oxidative stress [126].

Bloomer et al. (2007) also investigated the effects of a single bout of high intensity squats on markers of oxidative stress in 13 resistance trained males. Participants performed 15 reps of 70% 1RM on a Smith machine as well as a Wingate Anaerobic Test on a separate day. Blood was drawn prior to and immediately post exercise and tested for blood lactate, protein carbonyls (protein damage), malondialdehyde (lipid damage), and 8-Hydroxydeoxyguanosine (DNA damage). There were no significant differences in protein carbonyls between the Wingate and squat tests, but a significant increase over time ($p=0.04$, squatting 74% increase, Wingate 111% increase) [39]. There were no significant differences between exercises or over

time for malondialdehyde or 8-hydroxydeoxyguanosine (p>0.05). This study demonstrates that it is possible for a single set of anaerobic exercise to induce oxidative stress in resistance trained males, although only changes in protein carbonyls are seen immediately post exercise [39].

Güzel et al. investigated the effects of high- and low-intensity resistance training sessions on markers of oxidative stress and muscle damage in twenty healthy males [37]. Participants were randomly assigned to either a high-intensity, low-volume program (n=10) or a low-intensity, high-volume program (n=10). Exercises consisted of squats, leg extensions, lat pull downs, and chest press for both protocols. The 1RM for all lifts were tested one week prior to the exercise session. Blood was collected prior to and immediately after completion of the exercise circuit, as well as 6, 24, 48, and 72 hours post exercise. Nitric oxide (NO_x), thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), and creatine kinase (CK) were then assessed from these samples. Repeated measures ANOVA were used to assess differences between groups. Nitric oxide levels were significantly increased from 6 to 48 hours post-exercise (p<0.05 for all) in the high-intensity group, but did not change in the low-intensity group. Malondialdehyde was increased immediately post-exercise in both groups (p<0.05), but had returned to baseline by 6 hours post-exercise, however, the increase in the high-intensity group was significantly greater than that of the low-intensity group (p<0.05). Creatine kinase was significantly increased in the high-intensity group immediately post-exercise up to 48 hours post-exercise (p<0.05 for all) before returning to baseline. There were no significant differences between groups since the low-intensity group followed a similar course. Additionally, there was a significant correlation between CK

and NO_x in the high-intensity group (r=0.557, p<0.05). The authors concluded that high-intensity exercise increased NO_x, MDA and CK levels post exercise while low-intensity exercise only increased MDA and CK levels. This seems to indicate that increases in NO_x levels seemed to be caused by more intense resistance exercise [37].

Hoffman et al. examined the influence of exercise intensity on lipid peroxidation and what effect muscle oxygenation had on lipid peroxidation during resistance exercise in eleven resistance trained males [40]. Participants performed a 1RM squat prior to the study and on two subsequent visits performed 5 sets of either a low-intensity (15 reps at 60% of 1RM) or high-intensity (4 reps at 90% of 1RM) squats with 3 minutes between sets. Near infrared spectroscopy was used to measure muscular oxygenation in the vastus lateralis at rest, during each set and for 3 minutes post-exercise. Blood was drawn pre-exercise, immediately after, and 20 and 40 minutes post-exercise and assessed for malondialdehyde and blood lactate concentration. Two-way ANOVAs were used to analyze differences (time X group) using a Bonferroni post-hoc. Malondialdehyde increased significantly immediately post-exercise compared to pre-exercise and 40 minutes post-exercise in both groups with no significant difference between groups at any time point. Lactate concentrations increased post-exercise in both groups with the increase being significantly greater in the low-intensity compared to the high-intensity group (15.3±2.3 $mmol \cdot L^{-1}$ vs 9.7±3.3 $mmol \cdot L^{-1}$, p<0.05). There was also a significant correlation between the time it took to achieve 50% reoxygenation post-exercise and malondialdehyde concentrations immediately post-exercise (r=0.45, p<0.05) as well as the area under the curve for malondialdehyde (r=0.44, p<0.05). Malondialdehyde increased independently of exercise intensity following acute

resistance exercise, but appears to be significantly correlated with the rate of muscular reoxygenation following exercise. The authors suggest that this might be caused by the increases in tissue acidosis as indicated by decreased rate of deoxygenation and shifting of the oxygen hemoglobin disassociation curve to the left.

Rietjens et al. investigated the effects of resistance exercise on antioxidant activity, oxidative damage and inflammation in seven untrained males. One repetition maximum (1RM) testing for leg press and leg extension was performed a week prior to the study and didn't consume food rich in antioxidants for 3 days prior to data collection. Participants completed 8 sets of 10 repetitions at 75% 1RM for leg press and leg extensions with the starting exercise randomized. If participants were unable to complete all 10 repetitions the resistance was reduced to 65% or 55% 1RM (only one participant was able to complete all sets at 75% 1RM). Venous blood was collected prior to exercise (0 min), during the exercise (20 min), and at 43, 60, 90, 120,150, and 180 min post exercise as well as 24 hours post exercise and blood lactate, Total antioxidant capacity (TEAC), uric acid, vitamin C, vitamin E, and GSH were determined [38]. GST, GSH, uric acid and protein levels were also analyzed from muscle biopsies collected pre- and post-exercise. Blood plasma levels of lactate, TEAC, uric acid, vitamin C, and vitamin E increased during exercise. Uric acid, GST, and glutathione in muscle also increased following exercise. The authors concluded that a single session of resistance exercise induced oxidative damage in healthy males [38].

Hudson et al. attempted to quantify the changes in measures of blood oxidative stress following resistance training for both hypertrophy and strength. Ten subjects performed four sets of 10 reps of back squats at 75% of 1RM with 90 seconds of rest

between sets for the hypertrophy session and 11 sets of 3 reps at 90% of 1RM with 5 minutes of rest between sets for the strength session. These protocols were designed to have equal work performed in each session. Blood was collected before, immediately after exercise and one hour after exercise to analyze blood lactate, TEAC, uric acid, lipid peroxidation, and protein carbonyls. Both protocols created a significant rise in blood lactate levels immediately after exercise (p=0.05), but the increase following the hypertrophy session was substantially higher in the hypertrophy protocol (13.04±0.46 vs 7.13±1.19 mM). Plasma TEAC rose initially following the hypertrophy protocol, but returned to baseline at 60 minutes post exercise. Lipid hydroperoxides did not significantly change following either protocol, but did rise at 60 minutes post in the strength protocol, protein carbonyls increased threefold immediately following the strength and hypertrophy protocols and fivefold at 60 minutes post exercise. The authors concluded that both strength and hypertrophy squat programs created measurable oxidative stress in the blood as assessed by protein carbonyls [127].

Çakir-Atabek et al. investigated the oxidative stress response to different intensities of resistance training both acutely and after 6 weeks of training in 16 healthy young males [35]. Participants were randomly divided into either a hypertrophy (n=9) or strength training group (n=7). Both groups performed chest press, leg extension, lat pull-down, leg curl, shoulder press, and biceps curl. The hypertrophy group performed 3 sets of 12 reps of each exercise at 70% of estimated 1RM with 90 seconds between sets and the strength group performed 3 sets of 6 reps at 85% of estimated 1RM with 180 seconds between sets. Blood samples were obtained immediately prior to and immediately after resistance trained on the first day of training and on the first day of the

fourth and sixth weeks of training and analyzed for malondialdehyde, and reduced glutathione. Data were analyzed using paired *t*-tests with a Bonferroni correction. Strength was significantly increased for all exercises following 6 weeks of training in both the hypertrophy and strength ($p<0.05$). Malondialdehyde was significantly decreased immediately after hypertrophy training following the 4th and 6th weeks ($p<0.05$, 21-24%) and at all time points after strength training ($p<0.05$, 35-39%). The values from the first week were also significantly higher than the values from both the 4th and 6th weeks ($p<0.05$ for both). There were no significant differences between pre- and post-exercise reduced glutathione levels in either group at any time point ($p>0.05$). There was however a significant rise in reduced glutathione during the 6th week compared to the values at weeks 1 and 4 ($p<0.05$). The authors concluded that levels of malondialdehyde and reduced glutathione appeared to change regardless of training intensity [35].

Deminice et al. (2010) compared salivary biomarkers of oxidative stress and antioxidants before and after a resistance training session in 11 well-trained males as compared to traditional blood markers [36]. Participants performed 3 sets of 10 repetitions at 70% of 1 RM for bench press, cable pull down, overhead press, leg extension, leg flexion and leg press with 90 seconds of rest between sets. Venous blood samples were collected immediately prior to and 10 minutes after the exercise session. Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), advanced oxidation protein products (AOPP), lactate concentration, uric acid, total protein and total hydroperoxide were measured in plasma and saliva. Student's t-tests were used to compare differences in plasma and saliva samples pre and post

resistance training and correlations were run between salivary and blood samples. As expected, blood lactate and heart rate rose significantly with training (p<0.05). Blood samples showed significant increases in TBARS (42%), AOPP (27%), uric acid (26%), and GSH (14%) following acute resistance training (p<0.05). Only salivary uric acid also rose significantly with training (p<0.05). Uric acid was the only measure to have significant correlation (r=0.51, p<0.05) between blood and salivary measures (blood 26% increase, saliva 36% increase). The authors concluded that an acute session of resistance training increased oxidative stress in trained young men 10 minutes post exercise in blood markers, but not in salivary markers with the exception of uric acid, which often acts as a scavenger of reactive nitrogen species [36].

Deminice et al. (2011) compared the oxidative stress response to acute hypertrophy circuit resistance training and traditional hypertrophy interval training in 11 well trained participants [128]. This randomized cross over study consisted of one week of hypertrophy resistance traditional interval training (3 sets of 10 repetitions at 75% of 1RM with 90 seconds of rest between sets) and one week of hypertrophy-resistance circuit training (3 sets of 10 repetitions of 75% of 1RM alternating 2 different exercises with different muscle groups). Exercises consisted of bench press, cable pull down, overhead press, leg extension, leg flexion and leg press. Participants were tested prior to the start of the study, at the midpoint, and at the end of the study. At the midpoint and end of the study blood was drawn prior to and 10 minutes after the exercise session. Blood was then assessed for thiobarbituric acid reactive substances, reduced glutathione, total hydroperoxide, advanced oxidation protein products, vitamin E, uric acid, creatine kinase, total protein and blood lactate concentration. Paired student's *t*

tests were used to assess differences between training programs. There were no differences between programs for blood lactate or ratings of perceived exertion. Significant increases were seen in creatine kinase in both exercise programs (67% for traditional interval training and 33% for circuit training, $p<0.05$ for both). Only traditional interval training increased thiobarbituric acid reactive substances, reduced glutathione and uric acid (40, 14, and 25% respectively, $p<0.05$ for all). Hypertrophy-resistance interval training increased oxidative stress and promoted antioxidant modulation responses in trained individuals. The authors concluded that circuit hypertrophy-resistance training generates a lower oxidative stress response, which may be due to the decreased time needed to complete the session [128].

Cardosa et al. analyzed the effects of intermittent exercise and resistance exercise on blood cell counts and oxidative stress in 34 trained middle aged women [129]. Twelve women had a history of resistance training twice a week for at least the previous two years, another twelve women had engaged in spinning twice a week for at least the previous two years and the final ten women served as sedentary controls. The resistance group performed three sets of ten reps at 75-80% of 1RM for an 11 station circuit. The spinning group performed 50 minutes of intermittent aerobic exercise on a cycle ergometer at 70-85% of max HR. The control group rested for 2 hours. Blood samples were collected at rest, immediately after and 1 hour after exercise and analyzed for malondialdehyde, protein carbonyls, glucose, lipid profile, non-protein sulfhydryl, catalase and superoxide dismutase. Mann-Whitney tests were used for comparisons between groups and Wilcoxon tests were used for comparisons over time. Lipid peroxidation increased after exercise compared to rest in both groups

(p<0.05) and decreased again after 24 hours (p<0.05), but did not differ significantly from controls. Protein carbonyls increased in both groups immediately after exercise and after recuperation (p<0.05), but again were not significantly different from controls. Superoxide dismutase and catalase activity were significantly decreased after exercise and recuperation in both the resistance and spinning group (p<0.05 for both). The authors concluded that, like aerobic exercise, high intensity anaerobic and intermittent exercise results in oxidative stress and immune suppression in middle aged women. Additionally, trained individuals appeared to benefit from healthy blood lipid profiles and higher resting antioxidant levels available for limiting oxidative stress [129].

Goldfarb et al. (2012) investigated if Wingate cycle training would influence the oxidative stress response to an acute bout of cycling in nine active males [32]. Prior to training, participants performed a WAnT with blood drawn prior to and 1, 5, 10, 15, 30, and 90 minutes post exercise and analyzed for malondialdehyde, xanthine oxidase and protein carbonyls. Participants then trained 3 times a week for 3 weeks, performed 4 WAnT per session the first week, 5 per session the second week and 6 per session the last week. Peak power increased significantly with training while mean power did not. Malondialdehyde response did not significantly change with WAnT training but did decrease over time following aerobic cycling (p=0.08), xanthine oxidate did not change in response to acute aerobic cycling both before and after training (p>0.05). Protein carbonyls increased over time following acute exercise both with no change due to WAnT training (p<0.001). The WAnT increases oxidative stress with acute exercise but WAnT training did not influence these changes [32].

Kilic-Toprak et al. investigated the acute and chronic effects of 12 weeks of circuit resistance training on hemorheological parameters in twelve healthy sedentary males [64]. Participants performed a seven exercise circuit (leg extension, leg curl, chest press, arm flexion, arm extension, abdominal crunch, twisting oblique and outer thigh pull for 1 set of 8-12 reps at 40-60% 1RM during the first week, 2 sets during the second week and 3 sets during the third week, from weeks 4-12, 3 sets of 8-12 reps were performed at 75-80% 1RM. One repetition maximums were collected at weeks 0, 1, 2, 3, 5, 7, 9, 11, and 12 in order to adjust the resistance level. Maximal oxygen consumption was estimated using a treadmill and the Bruce protocol. Blood was collected before and after the first day of weeks 1 and 4, and the last day of weeks 3 and 12 and assessed for RBC deformability, RBC aggregation, whole blood and plasma viscosity, RBC count, hemoglobin and hematocrit, mean corpuscular volume, blood lactate concentration, plasma fibrinogen concentration, plasma total oxidant status, plasma total antioxidant status and oxidative stress index. There were no significant differences in anthropometric measurements or VO_{2MAX} over the course of the training intervention. Muscular strength had increased significantly by week 12. Aggregation of RBCs increased after the first day of training but then decreased throughout the protocol ($p<0.05$). Pre-exercise WBV hematocrit and plasma viscosity were decreased at weeks 4 and 12 ($p<0.05$) but increased post-exercise ($p<0.05$). Post-exercise WBV hematocrit was increased at week 12 ($p=0.01$). Fibrinogen levels were decreased after the third week but increased on the fourth week ($p<0.01$ and $p=0.05$, respectively). Plasma antioxidant status was increased at week three ($p=0.034$) and the oxidative stress index decreased at week four post-exercise ($p=0.013$). The authors concluded

that progressive resistance training may increase cardiovascular health by increasing RBC deformability, decreasing viscosities, and decreasing RBC aggregation as well as increasing antioxidant activity to reduce oxidative stress [64].

Scheffer et al. investigated the response of muscular resistance, hypertrophy, and strength training on metabolism and oxidative stress in 24 Wister rats [26]. Animals were distributed into 4 groups: control, muscular resistance training, hypertrophy training, and strength training. Training was conducted 4 days a week for 12 weeks. Blood was drawn following the first and last training session and the brachioradialis muscles were surgically removed for analysis following euthanasia. Muscle glycogen, superoxide production, superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde were analyzed. Muscle glycogen was increased in all training groups compared to controls. Superoxide production was increased in the hypertrophy and strength training groups compared to controls. There also was a significant increase superoxide dismutase and a decrease in catalase activity in the hypertrophy and strength training groups compared to controls. Glutathione peroxide increased in the hypertrophy and strength training groups compared to controls. Hypertrophy training increased oxidant levels, which led to an increase in oxidative stress. The authors concluded that intensity of effort during training is directly related to oxidant production and that a greater number of repetitions and a lower workload seemed to cause less damage [26].

Azizbeigi et al. (2013) investigated the effect of eight weeks progressive resistance training on erythrocyte antioxidant activity and oxidative stress in twenty young untrained males [34]. Participants were separated into either a control (n=10) or

exercise group (n=10). One repetition maximums were predicted for both upper and lower body exercises from a 3 repetition maximum in both groups before and after the exercise intervention. The exercise group trained with circuit training three times per week for eight weeks on nonconsecutive days at 50-80% of estimated 1RM. Training loads were reassessed during week four and training loads adjusted accordingly. Blood samples were taken prior to and within 72 hours of the eight week interval and assessed for total antioxidant capacity and malondialdehyde in plasma, and superoxide dismutase and glutathione peroxidase in erythrocytes. Two way repeated measures of ANOVA were used to assess differences between and within groups (time by group). There were no significant changes in total antioxidant capacity or glutathione peroxidase over time. There was a significant time by group interaction (p=0.014), but not time effect for superoxide dismutase, indicating that it increased due to the resistance training. This was also seen with malondialdehyde (time by group interaction p=0.030, time effect p=0.058). In the exercise group, squat increased by 43.4% (p=0.001) and chest press by 31.2% (p=0.001). The authors concluded that eight weeks of resistance training in untrained males resulted in decreased oxidative stress (decreased lipid peroxidation) and increased antioxidant capacity (increased superoxide dismutase) [34].

Bogdanis et al. investigated the changes in oxidative stress following an acute bout and 3 weeks of high-intensity training in eight physically active males [42]. For the acute exercise session, participants performed a WAnT as part of the baseline and then performed nine high-intensity training sessions over three weeks with 48-72 hours in between sessions. Venous blood samples were collected at rest and 30 minutes after

the first and last exercise sessions, as well as at 24 and 48 hours after these sessions. Both the initial and the last session included four 30 second sprints with 4 minutes of rest between sets. Blood samples were analyzed for creatine kinase activity, protein carbonyls, thiobarbituric acid reactive substances, total antioxidant capacity and catalase. Two-way repeated measures ANOVAs (training X time) were used to analyze differences. Most values peaked 24 hours post exercise at the start of the study with the exception of catalase, which peaked 30 minutes post exercise. After three weeks of high intensity training, markers of oxidative stress had decreased (protein carbonyls 13.3±3.7% and thiobarbituric acid reactive substances 7.2±2.7%, $p<0.01$ for both, $\eta^2=0.45\text{-}0.90$). Creatine kinase activity also decreased despite an increase in workload (10.9±3.6%). Markers of antioxidants, on the other hand, increased with training (total antioxidant capacity 38.4±7.2%, catalase 26.2±10.1%, and GPX 3.0±0.6%, $p<0.01$ for all, $\eta^2=0.45\text{-}0.80$). An acute session of high intensity training induced a 1.5-2.5 fold increase in markers of oxidative stress and antioxidants 24 hours after exercise. The authors concluded that short term high intensity training attenuated oxidative stress and up regulated antioxidant activity [42].

Cook et al. (2013b) investigated the effects of resistance training on endothelial function, inflammation, vascular remodeling and oxidative stress in young African American (n=14) and Caucasian (n=18) males [65]. Participants were analyzed before and after a 6 week resistance training protocol. At baseline and post-resistance training measures the participants performed a VO_{2MAX} test on a cycle ergometer and performed a 1RM bench press as well as having blood drawn. Serum and plasma were then analyzed for inflammation (TNF-α, IL-10), endothelial function and vascular remodeling

(sICAM, sVCAM, MMP-2 and MMP-9), and oxidative stress (8-isoprostane). Training consisted of three one hour session per week that exercised legs, back and biceps on one day and chest, shoulders and triceps on the next. Repeated measures ANOVA were used to assess between and within group differences. There was a significant decrease in MMP-9 and 8-Isoprostane in African Americans (p<0.001 and p=0.026 respectively) and a time X group interaction for MMP-9 and 8-Isoprostane (p=0.036 and p=0.014 respectively). The increase in bench press was significantly correlated with the decrease in MMP-9 (r=-0.398, p=0.022). The authors concluded that 6 weeks of resistance training reduces matrix remodeling proteins and oxidative stress in African American males [65].

Deminice et al. (2013) evaluated the effects of creatine supplementation on oxidative stress and inflammatory markers following acute sprinting in 25 well-trained males [23]. Testing was performed weekly for 3 weeks, anthropometrics were collected during the first visit and a running-sprint test (six 35 meter sprints with 10 seconds of rest) was conducted on weeks 2 and 3. Blood draws were performed before, immediately after, and one hour after the running-sprint test and analyzed for plasma creatine, malondialdehyde, reduced glutathione, oxidized glutathione, Ferric-reducing antioxidant power, creatine kinase, lactate dehydrogenase, tumor necrosis factor alpha and C-reactive protein, as well as erythrocyte glutathione peroxide, catalase, superoxide dismutase were determined. Participants then received one week of either placebo or creatine supplementation (0.3 $g \cdot kg^{-1}$) before the second running-sprint test. Creatine supplementation did not alter the oxidant, antioxidant, inflammatory, or blood lactate response to the running-sprint test. As expected, plasma creatine increased

201% with creatine supplementation, and improved running-sprint test performance compared to controls. Creatine supplementation inhibited increases in tumor necrosis factor alpha and C-reactive protein caused by acute exercise. The authors concluded that creatine supplementation does has anti-inflammatory effects but did not prevent oxidative stress [23].

Azizbeigi et al. (2015) investigated if the intensity of resistance training had any effect on antioxidant defense and inflammation in thirty untrained males [63]. Participants were randomly assigned to a moderate intensity, high intensity, or control group (n=10 for each). A 1RM for both bench press and leg press was completed prior to the intervention. Resistance training was completed three times a week for eight weeks and consisted of chest press, lat pull down, leg extension, leg flexion, biceps curls, triceps curls, squats, and sit-ups. Participants had their 1RM retested at the end of week 2, 4 and 6 in order to adjust the intensity of the program. The moderate intensity group performed 3 sets of 10-12 reps at 65-70% of 1RM with 1-2 minutes in between sets and the high intensity group performed 3 sets of 3-6 reps at 85-90% of 1RM with a 3-4 minute rest between sets. Blood was drawn before and after the exercise intervention and plasma was analyzed for malondialdehyde, superoxide dismutase, glutathione peroxide, tumor necrosis factor alpha, interleukin 6 and creatine kinase. Two-way repeated measures ANOVA were used to analyze differences (group X time). Resistance training caused a significant change in superoxide dismutase (p=0.009), glutathione peroxide (p=0.001) and malondialdehyde (p=0.003). Superoxide dismutase was shown to be elevated in both the high and moderate intensity groups (increase of 13.9%, p=0.029, and 16.1%, p=0.024, respectively). The same was true of

glutathione peroxide (high: increase of 26.1%, p=0.012, and moderate: increase of 22.3%, p=0.017). Malondialdehyde decreased significantly in both high and moderate intensity groups (decreases of 35.9%, p=0.011, and 33.5%, p=0.004, respectively). Superoxide dismutase and glutathione peroxide were significantly different between control and exercise groups, but not between exercise protocols. Malondialdehyde was significantly different following exercise intervention between exercise groups and controls, but not between exercise interventions. There were no significant group by time interactions for interleukin 6, tumor necrosis factor alpha and creatine kinase. Lipid peroxidation was not correlated with interleukin 6 and tumor necrosis factor alpha in either exercise group. The authors concluded that both exercise interventions were able to increase antioxidant defense and decrease oxidative stress [63].

Wiecek et al. (2015b) compared changes in antioxidants within 24 hours of anaerobic exercise in 10 men and 10 women [43]. Participants performed both a graded exercise test on a treadmill to determine VO_{2MAX} and a 20 second sprint on a cycle ergometer to determine peak and mean power. Blood was drawn prior to the sprint test, as well as 3, 15, 30, 60 minutes and 24 hours post-sprint test and then assessed for lactate concentrations, hydrogen ion concentrations, total oxidative status, total antioxidative capacity, Vitamins A, E, and C, uric acid, and reduced and oxidized glutathione. Differences between variables following sprinting was determined using MANOVAs and Tukey post-hoc tests. Both sexes showed the highest concentrations of total oxidative status, total antioxidative capacity, vitamin A, and vitamin E at 3 minutes post-sprint. There was also a significant decrease in reduced and oxidized glutathione at 15 minutes post-sprint in both sexes. The decrease in reduced glutathione lasted for

24 hours while the decrease in oxidized glutathione had turned into a significant increase by 1 and 24 hours post-sprint. This caused the ratio of reduced to oxidized glutathione to be significantly reduced at 1 and 24 hours post-sprint compared to resting values. Vitamin A and total antioxidative capacity were significantly higher in men, while women had significantly higher levels of vitamin C. Anaerobic exercise caused significant changes in antioxidants and markers of oxidative stress up to 24 hours post exercise. The authors concluded that anaerobic exercise caused the same changes in total antioxidative capacity, and total oxidative status, and glutathione that could last up to 24 hours post exercise [43].

The oxidative stress response to exercise is increased in men compared to women [15, 16], but the type of sport that an individual participates in does not effect on the oxidative stress response [17]. Training status, on the other hand, does affect this response, with trained individuals having a reduced oxidative stress response and increased antioxidant levels compared to untrained individuals [15, 18, 19]. Overtrained individuals display impaired antioxidant levels [20], and antioxidant supplementation blunts the increase in antioxidant levels seen acutely or with training [21, 22]. Creatine supplementation, while having anti-inflammatory properties, had no influence on antioxidant levels [23]. Cold temperatures were also shown to decrease antioxidant defense [24]. It has been postulated that the intensity of the exercise, rather than the type, was responsible for increased oxidative stress with a single bout of exercise [25, 26].

There are several studies with conflicting findings on exercise causing oxidative stress, with some studies reporting that oxidative stress increased following maximal

aerobic exercise [20, 27] while other studies reporting that it was unchanged [28, 29] and another reporting that there was a rise in antioxidants, but not oxidative stress [30]. Mixed aerobic and anaerobic training, such as that for Muay Thai, increased oxidative stress during training and competition [62]. The evidence for oxidative stress with anaerobic exercise is more consistent, with the Wingate Anaerobic Test [31-33] and resistance training [34-40] shown to increase oxidative stress following a single bout of exercise. Isometric and eccentric resistance exercise has also been shown to increased oxidative stress [22, 41]. Training blunted this response and increased antioxidants in circuit training [34, 63, 64] and traditional resistance training [65], but the response in the Wingate Anaerobic Test is conflicted [32, 42].

One potential reason for the differences between findings in this research is the multitude of time points and markers utilized when investigating oxidative stress. Since the Reactive Oxygen Species are transient by nature due to their high reactivity, it is difficult to measure them directly; and their effects on other substances (proteins, lipids, DNA) or the following increase in antioxidants to counteract them is measured instead. Additionally, these substances peak at different time points post exercise (some up to 24 hours), meaning that some studies may have missed significant changes [42, 43].

Oxidative stress has been shown to increase following a single session of the back squat exercise and the level of oxidative stress that occurs (specifically in lipid peroxidation) appears to be related to rate of tissue reoxygenation [39, 40]. It is possible that the use of low intensity exercise with blood flow restriction could therefore increase the acute oxidative stress response more than that seen with traditional resistance training. Although previous studies investigating oxidative stress with BFR

have shown mixed results [46, 47], neither utilized a multi-joint exercise such as the squat and the time points between the two studies differed.

Fourier Transform Infrared Spectrophotometry

Fourier-transformed infrared spectrophotometry has previously been utilized for the quantification of several different substances in both blood and saliva. Additionally, these protocols can allow for the rapid assessment of multiple substances from a small sample, which, in the case of blood, can be collected from a free-flowing digit puncture, rather than a venous puncture. This may allow for the assessment of multiple measures without utilizing tests that require expensive reagents and are easily run.

Oxidative Stress in Erythrocytes

Shaw et al. attempted to determine concentration levels of eight analytes in blood serum (total protein, albumin, triglycerides, cholesterol, glucose, urea, creatinine and uric acid) in 300 serum samples [130]. An internal standard of 1 mL of 4 $g \cdot L^{-1}$ of aqueous potassium thiocyanate was added to each serum sample. Seven microliters of this diluted serum was then spread on a BaF_2 window and allowed to dry for 30 minutes before being analyzed for spectra using 256 scans at 4 cm^{-1}. Spectra were then analyzed with a partial-least squares method and assessed for variance that could be attributed to the FTIR collection method. The optimized calibration models for each analyte contained the following spectra: albumin (1100-1800), cholesterol (1100-1300, 1700-1800, 2800-3000), glucose (925-1250), total protein (900-1800), triglycerides (900-1500, 1700-1800, 2800-3000), urea (1400-1800), total CO_2 (900-1500), creatinine (800-1600, 2800-3500), and uric acid (800-1800, 2800-3500). The authors considered six of the eight analytes (albumin, cholesterol, glucose, total protein triglycerides, and

urea) to be able to be measured precisely and accurately when analyzed using FTIR spectrophotometry [130].

Petibois and Déléris (2004) used FTIR to determine the effects of oxidative stress from 120 min of aerobic exercise on erythrocytes in 16 trained male individuals [48]. Participants performed 2 hours of cycling at 55% of their maximum power output with VO_2, VCO_2, and RER calculated throughout exercise. Capillary blood samples of approximately 100 microliters were taken at rest and every 15 min during exercise. The samples were then centrifuged for 3 min at 15000 g within one minute of collection and the separated plasma was frozen at -20° C prior to analysis within 48 hours. Erythrocytes were collected from below the gel-barrier and washed with saline prior to being centrifuged again three times. Erythrocytes were then stored at 4° C prior to analysis. Both samples were diluted prior to dehydration and analysis; 20 µL of plasma were diluted with 80 µL of water and 10 µL of erythrocytes were diluted with 190 µL of water. Thirty-five microliters of the sample was then placed on zinc-selenide glass and desiccated in a vacuum dryer. Spectra were collected for FTIR analysis between 4000 and 400 cm^{-1} using 32 scans at a resolution of 2 cm^{-1} and a beam diameter of 6 mm. Spectral absorbances were corrected for level of Hct change prior to analysis. Plasma glucose increased at the start of exercise (p=0.03) and decreased further at 105 minutes (p=0.01). This pattern was also followed by plasma lactate (p=0.001 for the increase, and p=0.02 for the decrease at 120 min). Triglyceride concentrations increased 25% during the first hour of exercise (p=0.05) and increased further at 90 minutes (p=0.01) while fatty acid moieties increased after 75 min (p=0.03) and further at 105 min (p=0.02). Oxygen uptake increased with the start of exercise (p=0.001) and

remained level until 90 min, at which point they increased further at 120 min (p=0.001). Interestingly, lactate concentrations were found to increase 2-fold in erythrocytes and 4-fold in plasma over the course of the study. Absorbance for $v_{as}(CH_3)$ (2880-2860 cm^{-1}) remained stable throughout activity (p>0.10) while $v_{as}(CH_2)$ (22880-2950 cm^{-1}) remained stable for 90 minutes (p>0.10) before decreasing significantly (p=0.003). This led to the CH_2/CH_3 ratio following a similar pattern, decreasing at 90 min (p=0.01). The v(C=O) (1739-1713 cm^{-1}) and δ(N-H) (1589-1474 cm^{-1}) absorbance decreased after 90 min (p<0.05). The v(P=O) (1257-1201 cm^{-1}) absorbance decreased after 45 min (p=0.03) and decreased even further at 120 min (p=0.02). The $v_{as}(CH_3)$, v(C=O), δ(N-H), v(P=O) absorbances and CH_2/CH_3 ratio all paralleled the increase in VO_2 (p<0.001). The authors used spectral area under the curve was used since the authors believed it to be the most informative about erythrocytes. Since the $v_{as}(CH_3)$, $v_{as}(CH_2)$, and v(P=O) bands are only present in fatty acyl chains in the erythrocyte, The authors have used these as a measure of the changes to the phospholipid membrane of the cell. The v(C=O) and δ(N-H) bands are representative of proteins in the erythrocyte, of which 90% is hemoglobin (10% spectrin). Any changes in these bands are most likely due to denaturation of hemoglobin and the v(C-O) (1138-1061 cm^{-1}) band was associated with lactate concentration. The authors concluded that phospholipid peroxidation occurred as indicated by the changes in the $v_{as}(CH_2)$ and v(P=O) bands and that FTIR spectrophotometry could be useful for determining acute plasma and erythrocyte parameters associated with moderate, physiological oxidative stress [48].

Petibois and Déléris (2005a) also investigated the effects of 19 weeks of aerobic training on oxidative stress in erythrocytes in fifteen male rowers following a 6 week off-

season [49]. Participants performed a VO_{2MAX} test at weeks 1, 6, 12, and 24. Training consisted of rowing for 120 km per week at 65-75% of VO_{2MAX}, with a standardized rowing session performed in the lab each week to adjust training intensity. Capillary blood samples were collected before and after each standardized session and processed and analyzed as described previously [48, 50]. Levels of superoxide dismutase (SOD) were also measured. Levels of SOD increased following 5 weeks of training ($p=0.04$) and again after 14 weeks ($p=0.03$). No changes were seen in the levels of SOD between exercise and rest throughout the study. While resting plasma values did not change throughout the study, changes due to exercise differed significantly after 6 weeks of training. Following that, post exercise fatty acid moieties increased at 15 weeks ($p<0.01$) and plasma volume change decreased after the 13^{th} week ($p<0.01$). Plasma volume change was inversely correlated with training experience ($p<0.05$). All post-exercise erythrocyte spectra had significantly changed following 6 weeks of training ($p<0.05$). Following that change the $\nu(CH_n)$ band for phospholipids and fatty acids (2996-2795 cm^{-1} increased at week 10 ($p<0.05$) and the protein bands, $\nu(C=O)$ and $\delta(N-H)$ (1695-1574 cm^{-1} and 1574-1471 cm^{-1} respectively) also increased in the same manner ($p<0.05$). The absorption for $\nu P=O$ increased again at 15 weeks (greater than week 6, $p<0.05$), while the absorption for erythrocyte lactate decreased over the same time period. Training level was significantly correlated with all erythrocyte parameters except νCOO^- ($p<0.02$). All correlations were positive with the exception of erythrocyte lactate. Plasma volume change with exercise had an inverse correlation with $\nu C-(H)_n$, $\nu C=O$, $\delta(N-H)$, and $\nu P=O$ ($p<0.02$ for all), and a positive

correlation with erythrocyte lactate ($p<0.01$). The authors concluded that endurance training enhanced erythrocyte oxidative stress defense as measured by FTIR [49].

Petibois and Déléris (2005b) investigated the amount of oxidative stress that occurring in erythrocytes during high intensity aerobic cycling in 21 trained participants [50]. A 120 min cycling exercise was performed starting at 50% of VO_{2MAX} and increasing to 75% of VO_{2MAX} by the end of the session. Capillary blood samples of about 100 µL were drawn at rest and every 20 min during the study. The samples were then centrifuged for 3 min at 15000 g within one minute of collection and the separated plasma was frozen at -20° C prior to analysis within 48 hours. Erythrocytes were collected from below the gel-barrier and washed with saline prior to being centrifuged again three times. Erythrocytes were then stored at 4° C prior to analysis. Both samples were diluted prior to dehydration and analysis; 20 µL of plasma were diluted with 80 µL of water and 10 µL of erythrocytes were diluted with 190 µL of water. Thirty-five microliters of the sample was then placed on zinc-selenide glass and desiccated in a vacuum dryer. Spectra were collected for FTIR analysis between 4000 and 400 cm^{-1} using 32 scans at a resolution of 2 cm^{-1} and a beam diameter of 6 mm. Spectral absorbances were corrected for level of Hct change prior to analysis. Plasma lactate increased significantly during the first 80 min of exercise ($p=0.01$) and then decreased shortly thereafter ($p=0.001$). Plasma triglycerides increased slowly at first ($p=0.02$) and increased further at 80 min ($p=0.02$) while fatty acid moieties only increased after 80 min ($p=0.05$). As before, the absorbance of $v_{as}(CH_3)$ remained stable throughout exercise ($p>0.10$) while $v_{as}(CH_2)$ decreased linearly after 40 min ($p=0.003$). Thus the CH_2/CH_3 ratio decreased after 40 min of exercise ($p=0.01$). The absorbance of v=(CH)

increased after 40 min (p=0.003). The v(P=O) absorption decreased after only 20 min of exercise (p=0.03). Protein v(C=O) absorption increased after 80 min of exercise (p=0.001) while δ(N-H) decreased linearly after 40 min ($p<0.05$). Lactate v(C-O) absorption increase slightly with exercise (p=0.001) but increased more pronouncedly towards the end of exercise (80-120 min, p=0.002). The authors attributed the significant changes in v(C=O) and δ(N-H) to hemoglobin denaturation due to due to oxidative stress and the changes in phospholipids as damage to the cell membrane from peroxidation from oxidative stress [50].

Concentrations of Molecules in Plasma

Petibois et al. (1999) attempted to measure the absorption of glucose with FTIR in 32 diabetic participants [57]. Venous blood was drawn and centrifuged for 10 min at 4000 *g*. The sample was then diluted with saline to different serum to saline concentrations (15:85, 20:80, and 25:75). Then 35 microliters were placed on a zinc-selenide disc and placed in a drying vacuum to evaporate water. The sample was then analyzed in a FTIR spectrophotometer from 400 to 4000 cm^{-1} with 32 scans at a resolution of 2.0 cm^{-1} and a beam diameter of 6 mm. The 20:80 dilutions had the lowest variation in spectra between samples and were used for further analysis. Glucose absorption was determined using known glucose concentrations of 6.77, 5.44, 2.76, and 1.38 $mmol \cdot L^{-1}$. Spectral analysis indicated that the peak between 997 and 1062 cm^{-1} had the highest reproducibility (mean difference 1.76%). Glucose concentration per unit of spectral area (U) was determined to be 7.27 $mmol \cdot L^{-1} \cdot U^{-1}$. Analysis of glucose via FTIR had a strong correlation with glucose assessed by traditional methods (r=0.998, $p<0.01$). This led to a regression model with glucose concentration determined by

traditional methods that had a slope of 1.006 and an intercept of -0.049 mmol·L^{-1}. The authors concluded that spectral analysis of glucose using FTIR allows for accurate analysis of blood glucose [57].

Petibois et al. (2000) investigated the use of Fourier-transform infrared spectroscopy in the assessment of lactate concentrations using small samples of blood [56]. Capillary blood samples of 50 microliters were taken at rest and after ~1.5 hours of high intensity rowing exercise (n=82). Blood was then centrifuged in a gel-separator tube for 3 minutes at 15,000 *g* prior to freezing at -20° C and was analyzed within 48 hours. Twenty microliters of serum was then diluted with eighty microliters of saline and homogenized. Then 35 microliters were placed on a zinc-selenide disc and placed in a drying vacuum to evaporate water. The sample was then analyzed in a FTIR spectrophotometer from 400 to 4000 cm^{-1} with 32 scans at a resolution of 2.0 cm^{-1} and a beam diameter of 6 mm. Lactate and glucose concentrations were also assessed via standard methods for comparison. Known concentrations of glucose and lactate solutions were also assessed by the FTIR in order to create a regression model. Lactate concentrations were found to have a high correlation ($r=0.97$, $p<0.001$) with the peak located at 1127 cm^{-1} and the specific absorption at the band was 3.67 mmol/L/U. Since glucose and lactate absorb at similar wavelengths, glucose concentrations were subtracted from the serum spectra prior to analysis for lactate which resulted in a regression with lactate analyzed via traditional measures with a slope of 1.02 and an intercept of 0.08. This method of subtraction of known spectra prior to analysis can potentially be used for analysis of other substances as well [56].

Petibois and Déléris (2003) investigated the use of FTIR in assessing changes in plasma volume with exercise [88]. Blood samples were drawn at rest and after exercises of various intensities and durations and compared against plasma volume (PV) change as assessed via hematocrit (Hct) using the following equation: $\%\ PV\ Change = \frac{100}{100-Hct_{Pre}} * \frac{100*(Hct_{Pre}-Hct_{Post})}{Hct_{Rest}}$. Samples for FTIR analysis were centrifuged in a gel-separator tube for 3 minutes at 15,000 *g*. Twenty microliters of serum was then diluted with eighty microliters of saline and homogenized. Then 35 microliters were placed on a zinc-selenide disc and placed in a drying vacuum to evaporate water. The sample was then analyzed in a FTIR spectrophotometer from 4000 to 500 cm^{-1} with 32 scans at a resolution of 2.0 cm^{-1} and a beam diameter of 6 mm. Total spectral area was found to be highly correlated with rest and post-exercise Hct levels (r=0.95 and 0.96, respectively, *p*=0.01 for both). The regression line between PV change and total spectral area had a slope of -0.93 and an intercept of 1.14. The equation: Exercise Plasma spectrum – 1.14 * Resting Exercise spectrum was then used to correct for changes in plasma volume during exercise. The authors concluded that the total area of the plasma FTIR spectra is useful for determining PV changes determined by exercise [88].

Cortisol in Saliva

Aardal and Holm attempted to establish morning and evening reference ranges for cortisol in saliva from samples collected from 197 healthy volunteers [131]. At least one morning and one evening saliva sample after taking nothing by mouth for one hour, followed by a venous blood draw. Ten participants also completed two additional sample collections over three weeks. Cortisol was measured using a solid phase

radioimmunoassay kit with reference ranges of 200-800 nmol·L^{-1} for morning values and <300 nmol·L^{-1} for evening values. Differences between serum and salivary collection methods were assessed using unpaired Mann-Whitney U tests. The intra-assay coefficients of variation was calculated at 4.3% for 0.5-10.0 nmol·L^{-1} and 3.6% for 10.1-123.0 nmol·L^{-1}. Morning and evening means and ranges were calculated for each age group and gender (21-30, 31-40, 41-50, 51-60, and 61-70 years old). Central ranges where estimated to be 3.5-27.0 nmol·L^{-1} at 8 AM and <6.0 nmol·L^{-1} at 10 PM. The authors concluded that salivary cortisol measurements are satisfactorily precise and can be used instead of serum cortisol measurement [131].

Paccotti et al. investigated the inter-individual differences in the lactate and cortisol responses to an acute bout of high intensity isokinetic exercise and examined the relationship between lactate, serum cortisol and salivary cortisol in 20 competitive and 11 non-competitive male athletes [132]. Each participant performed four sets of 20 maximal isokinetic knee flexions and extensions at 180°·sec^{-1} with a 30 second rest between sets and a 3 minute rest between legs. Blood and saliva were collected for cortisol evaluation 15 and 5 minutes prior to exercise, and at 0, 7, 15, 30, 45, 60, 90 and 120 minutes following exercise. Blood lactate levels were analyzed 15 and 5 minutes prior to exercise and 0, 30, 60 and 120 minutes after exercise. Saliva was also collected at various times of day (8 AM, 4 PM, 12 AM) from a large group (110 individuals, 58 men and 52 women) in order to establish a reference range for salivary cortisol at different time-points throughout the day. Mann-Whitney U tests were used to compare differences between collection methods and groups. Spearman's rank correlation was used to compare lactate and cortisol levels. Serum cortisol was

significantly higher in the competive athlete group at 30 and 120 minutes post exercise compared to non-competitive athletes (p<0.05) while salivary cortisol was higher 0, 90 and 120 minutes post-exercise in competitive compared to non-competitive athletes (p<0.05) and was higher in power athletes compared to endurance athletes (p<0.05). There was a significant difference in the slope of the regression line for values of serum cortisol over 500 nnmol·l^{-1} (0.017 vs. 0.037, p<0.01). Following logarithmic transformation there was also a significant correlation (r=0.62, p<0.001). Peak levels of serum cortisol were reached at 15 and 30 minutes post-exercise while peak levels of salivary cortisol were reached at 60 minutes post-exercise. Mean values for salivary cortisol were 20.4±7.7 nmol·l^{-1} at 8 AM, 9.2±4.3 nmol·l^{-1} at 4 PM and 5.5±3.1 nmol·l^{-1} at midnight. There were significant differences between time of collection (p<0.001). The authors concluded that the cortisol response to maximal exercise can be measured reliably and non-invasively using serial saliva sampling [132].

Poll et al. compared two methods for obtaining saliva for cortisol measurement compared to free serum cortisol and total serum cortisol in ten healthy volunteers [53]. Samples were collected on two consecutive days at 8 am, 11 am, 2 pm, 5 pm, 8 pm, and 11 pm. The sequence of saliva collected was changed on the second day. Salivary cortisol was measured via ELISA and free and total serum cortisol were determined using an immunoassay. Statistics were performed using a tree-way repeated measures ANOVA (Type X Day X Time). Salivary cortisol measurements were consistently significantly lower when collected using a gauze salivette compared to drooling into the collection tube (p=0.032). There were stronger correlations between the gauze collection and serum measurements (total serum cortisol r=0.813, free serum

cortisol r=0.836) than between the drool collection and serum measurements (total serum cortisol r=0.735, free serum cortisol r=0.751). By time point, the gauze method was significantly correlated with the serum measurements more often than the drool method. The gauze method was also a significant predictor of total and free serum cortisol at 8 of 12 time points while the drool method was only a significant predictor at 1 of 12. Participants and laboratory technicians also preferred the gauze collection method. The authors concluded that the gauze collection method is a reliable laboratory diagnostic tool [53].

Khaustova et al. (2009) aimed to analyze saliva contents using FTIR collected with attenuated total reflection (ATR) in 28 volunteers [52]. Saliva samples were collected using a cotton pad which was then spun at 3000 rpm for 20 min. One part of the sample was tested for cortisol, protein concentrations, glucose, salivary amylase, urea, and non-organic phosphate using traditional methods while the other portion was assessed using a FTIR spectrophotometer fitted with an ATR accessory. Two microliters of saliva were placed on the ATR and allowed to dry for 3 min. Spectra were recorded from 4000 to 700 cm^{-1} using 32 scans at a resolution of 4 cm^{-1} with air used as the background spectra. Each analysis was repeated 10 times with the ATR cleaned with distilled water between samples. The calibration models for sIgA had an R^2 of 78.2% and 79.4% for cortisol. Glucose had an R^2 of 84.6%, and phosphate had an R^2 of 87.2%. The authors concluded that a 10 µL sample of saliva could be utilized for analysis of several molecules in saliva with similar precision and reproducibility to routine clinical analysis [52].

Khaustova et al. (2010) then went on to adapt the FTIR analysis of saliva to simultaneously measure α-amylase, cortisol, sIgA, urea, total protein and phosphates and measure the changes that occurred with a treadmill ramp test in 48 trained males [51]. Participants performed a graded exercise test on a treadmill until exhaustion with saliva samples collected before, immediately after, and 30 minutes after exercise. Anaerobic threshold and maximal oxygen consumption were also determined. Saliva was collected with a cotton swab which was subsequently centrifuged to remove the saliva. A total 154 samples were collected, 120 were used for development of the model and the remaining 34 were used for validation. One half of the sample was used for routine biochemical analysis while the other half was used for FTIR analysis. A 2 microliter sample of saliva was placed on the attenuated total reflectance accessory and allowed to dry for three minutes. Spectra were then recorded from 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans using attenuated total reflectance and air was used as the background spectrum. The model for calculation of total protein level used the 1503-1440, 1317-1249, and 1190-936 cm^{-1} spectral ranges. Saliva sIgA concentrations used the 1567-1526 and 1488-1406 cm^{-1} spectral ranges. Cortisol concentrations were best determined using the 1943-1526, 1391-1249 cm^{-1}, and 1115-973 cm^{-1} spectral ranges. Salivary α-amylase concentrations were determined using the 1578-1548, 1526-1496, and 1444-1305 cm^{-1}. All parameters were found to have a high R^2 for both calibration (R^2=0.86-0.96) and validation (R^2=0.83-0.95). Cortisol was found to be significantly increased immediately after and 30 minutes after exercise (p<0.05) and this increase was significantly greater in individuals that were less fit (p<0.05). The authors concluded that attenuated total reflectance FTIR can accurately measure α-amylase,

cortisol, sIgA, urea, total protein and phosphates in saliva [51]. Madarame et al. (2010a) compared growth hormone and other marker responses to low intensity resistance training with BFR in upper and lower limbs for nine resistance trained males [54]. Participants completed two sessions; one each for upper- (biceps curl and triceps push down) and lower- (leg extension and leg curl) extremity training. Both protocols consisted of 4 sets of each exercise (one set of 30 repetitions, followed by three sets of 15 reps, with 30 seconds in between sets), performed at 30% of 1RM. Compression was achieved using 3 cm cuffs for the upper-arm and 4 cm cuffs for the upper-leg, inflated to 130 and 200 mmHg respectively. Blood samples were collected before exercise and 0, 15, and 30 minutes following completion of the exercise session and analyzed for lactate noradrenaline, growth hormone, testosterone, cortisol, and IGF-1. Repeated measures ANOVAs were utilized to analyze the data. There were no significant program, or time X program differences for lactate, noradrenaline, growth hormone, testosterone, or IGF-1; although all increased significantly with exercise ($p<0.05$ for all). The area under the curve for growth hormone was significantly greater with lower extremity BFR exercise ($p=0.025$). Serum cortisol levels only increased with lower-extremity BFR exercise ($p<0.018$) but not upper-extremity BFR. The authors concluded that while upper- and lower- extremity BFR exercises caused similar changes in lactate, noradrenaline, testosterone, and IGF-1, lower-extremity BFR exercise created a significantly larger increase in growth hormone [54].

Assessment of saliva and blood (plasma and erythrocytes) using FTIR appears to be a valid method of several markers of interest for physical exercise. This tool appears to be useful in cheaply measuring multiple substances in bodily fluids (saliva,

blood, plasma, etc.) without requiring a blood draw and can be used to accurately measure oxidative stress in erythrocytes and cortisol in saliva [48-52, 55-57]. Salivary cortisol collected with gauze is a reliable method of evaluating cortisol levels [53]. Serum cortisol has been shown to increase acutely with low intensity resistance training with BFR [54]. To date, no study has utilized FTIR to examine oxidative stress and salivary cortisol levels in erythrocytes with resistance training.

There seem to be several gaps in the literature: the use of FTIR to measure oxidative stress in erythrocytes with resistance training, the acute and chronic effects of low intensity BFR training on the oxidative stress responses in erythrocytes, the acute and chronic salivary cortisol response to pBFR training, and the EPOC response to low-intensity BFR training all have yet to be investigated. These areas could be investigated with the use of a single study comparing the acute response to both low intensity BFR training and traditional resistance exercise before and after a short-term practical BFR training intervention. An additional comparison could be made by including trained and untrained males in order to examine whether the training intervention can change the oxidative stress response in trained individuals.

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Appendix A: Informed Consent

Subject Information and Informed Consent Form:

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Phone Number:	808-956-9585 Off hours: 808-956-5555
Study Sponsor:	Kinesiology and Rehabilitative Science Department 1337 Lower Campus Rd, PE/A 231 Honolulu, HI 96822
Protocol Title:	Erythrocyte Oxidative Stress and Oxygen Consumption with Low-Intensity Blood Flow Restricted Resistance Exercise and Training
Protocol Number:	2016-30768
Date of Protocol:	

INTRODUCTION TO RESEARCH STUDY

You have been asked to take part in a research study

This Subject Information and Informed Consent Form tells you about the study. The researcher will go over this with you and answer any questions you may have regarding the study. Ask your researcher to explain any words or information in this consent form you do not clearly understand. You should understand the purpose of the study, how taking part may help you, any potential risks to you, and what is expected of you during the study.

If you agree to take part, you will be asked to sign and date this consent form and will be given a signed and dated copy to keep. No one can force you to take part in this study. Even if you agree to take part now, you can decide otherwise and stop at any time without penalty or loss of benefits to which you would otherwise be entitled.

PURPOSE OF THE STUDY

The purpose of this study is to look at the oxidative stress response in red blood cells following low-intensity resistance training with practical Blood Flow Restriction and if this effect is changed with two weeks of resistance training. Additionally, this study will compare the oxygen consumption following two types of resistance training (traditional high-intensity vs. low-intensity with practical BFR).

You have been asked to participate in this study because you are between 18 and 35 and have either not been performing regular resistance training for the past three months, or have not been performing regular resistance training for the past three months and are considered as low risk for exercise as determined by the criteria set forth by the American College of Sports Medicine. You will be excluded from the study if you have diagnosed hypertension, arrhythmia, ischemic changes in the heart, a BMI greater than 30 $kg \cdot m^{-2}$, or meet any of the absolute or relative contraindications for resistance training and testing established by the American College of Sports Medicine.

DESIGN OF THE STUDY

If you agree to participate, you will be one of about 30 participants recruited from the University of Hawaii at Mānoa community. This study is open to male participants, between 18 and 35 years of age, who meet the study requirements.

Once you are found to be eligible to participate in the study, and you state that you want to take part in the study, you will be assigned a subject number.

DURATION OF THE STUDY

Once you are found to be eligible to participate in the study (during the Initial Visit), and you state that you want to take part, your participation will last for a minimum of 5 weeks (about 1 month), including the Initial visit. During this time, you will be required to visit the lab at least 9 times (initial visit, two pre-training testing sessions, four training sessions, one post-training testing session, and final visit).

SUBJECT RESPONSIBILITIES

If you decide to participate, there are certain rules you must follow before, during, and after the study period. Some are listed below, but there could be others that the study doctor will discuss with you:

It is very important that you tell your researcher all of the information you know about your health and medications you may be taking throughout the study period. If you do not tell the researcher everything you know, you may be putting your health at risk.

You must follow all instructions given to you while you are participating in this study. If you do not, you may be removed from the study. If you are unsure about what you are supposed to do, ask the researcher.

STUDY PROCEDURES

Initial Visit

To help the researcher find out if you can participate, and to establish the level of resistance to be used in the study, you will need to perform an initial visit. After you sign the informed consent form and receive a copy of it, you will have several screening procedures done. Note that all of the procedures listed below may not be performed if at any point during the evaluation you fail eligibility. These procedures will include:

- An interview about your medical history
- A questionnaire about your health and if you are ready for physical activity
- A questionnaire about your ability to perform physical exercise
- Weight, height and vital sign measurement (blood pressure, thigh girth and skinfold measurements)
- A three repetition maximum for the back squat to estimate your bask squat one repetition maximum (if you are unfamiliar with the back squat, instruction will be provided)

The researcher will review all of your medical information and findings from your Initial Visit and other entry criteria, as required by the study protocol, to ensure that you are eligible to participate in this study before measuring your weight, height, and vital signs and conducting the three-repetition maximum back squat. During the blood flow restricted resistance training exercise protocols, elastic knee wraps will be applied to your upper thigh at a tightness of 7 out of 10 (tight, but not painful). After the cuff has been applied, the researcher will ensure that you still have blood flow in your legs. The cuff will be adjusted between sets if necessary to maintain the tightness of 7 out of 10.

Restrictions During the Study

You will be asked to not eat or drink anything except water for at least one hour before the Testing Sessions. Additionally, you will be asked to avoid non-steroidal anti-inflammatory medications, alcohol, tobacco, supplements and other medication if possible for 3 days before each of the Testing Sessions. You should check with the researcher about any medication or health supplements you are taking during the study.

Testing Sessions

One week after the Initial Visit, you will be asked to come back to perform one of two testing sessions, with the other session completed within a week. At this visit it is

important that you have eaten or drank nothing but water for one hour prior to your visit.

Procedures at this visit will include:

About 200 µL (about 4 drops) of blood will be collected from a free-flowing digit puncture taken at several points throughout the visit:

- Prior to exercise
- Immediately after exercise
- 30 minutes after exercise
- You will also be asked to come in the next day for a blood draw at 24 hours post exercise

Approximately 5-10 mL (1-2 teaspoons) of saliva will be collected from cotton balls that you have chewed on at three points during the visit

- Prior to exercise
- Immediately after exercise
- 30 minutes after exercise

Metabolic data will also be collected during exercise and for 30 minutes following exercise. In order for this to occur, you will be fitted with a mask to collect your expired air during and after testing.

The exercise protocol will be one of the following:

- Four sets of eight repetitions at 70% of estimated one repetition maximum
- One set of 30 repetitions, followed by three sets of 15 repetitions at 30% of estimated one repetition maximum with venous blood flow restriction

Training Sessions

You will be asked to come in four times over the course of two to three weeks for additional training sessions of resistance training using 30% of your estimated one repetition maximum with practical Blood Flow Restriction. These will be performed in the same manner as during the testing session, but without the blood draws before and

after, and no metabolic data will be collected.

Post-Training Testing Visit

At this visit it is important that you have eaten or drank nothing but water for one hour prior to your visit. Following the last of the training sessions you will come in for a final session of low-intensity resistance training with practical BFR. It will be similar to the Initial Testing session, but metabolic data will not be collected. Procedures at this visit will include:

About 200 µL (about 4 drops) of blood will be collected from a free-flowing digit puncture taken at several points throughout the visit:

- Prior to exercise
- Immediately after exercise
- 30 minutes after exercise
- You will also be asked to come in the next day for a blood draw at 24 hours post exercise

Approximately 5-10 mL (1-2 teaspoons) of saliva will be collected from cotton balls that you have chewed on at three points during the visit

- Prior to exercise
- Immediately after exercise
- 30 minutes after exercise

The exercise protocol will consist of one set of 30 repetitions, followed by three sets of 15 repetitions at 30% of estimated one repetition maximum with venous blood flow restriction.

Final Visit

Within a week of the last testing visit, you will be asked to come in one more time.

Procedures at this visit will include:

- Weight, height and vital sign measurement (blood pressure, thigh girth and skinfold measurements)
- A three repetition maximum for the back squat to estimate your bask squat one repetition maximum (if you are unfamiliar with the back squat, instruction will be provided)

RISKS

Back Squat Exercise

The back squat exercise utilized for this study may result in delayed onset muscle soreness following the first few visits. Once you become accustomed to the exercise load, you should not have any more increased soreness for the remainder of the study. During the back squat, your form will be monitored by the researcher and any deviations from the proper form will be corrected prior to the next repetition. It is still possible that some musculoskeletal injury may occur, but the intensity in the majority of sessions is rather low to help reduce this risk. You may experience delayed onset muscle soreness following resistance exercise, this should subside as you continue resistance training. The first three visits will be separated by one week to allow soreness to subside.

Practical Blood Flow Restriction

Serious side effects for BFR training include venous thrombus (0.055%), pulmonary embolism (0.008%), rhabdomyolysis (0.008%), and deterioration of ischemic heart

disease (0.016%) have been reported in Japan. Other, less serious side effects were reported that occurred more frequently than the serious side effects and included: subcutaneous hemorrhage (13.1%), numbness (1.297%), cold feeling (0.127%), pain (0.040%), itch (0.024%), feeling sick (0.016%) and increased blood pressure (0.016%). The cuffs used when reporting these side effects were narrower and placed a higher occlusive pressure on the limb.

Blood Sample Collection

In addition to risks linked with the study, drawing capillary blood may cause local pain, bruising, occasional lightheadedness, fainting, and very rarely, infection at the site of the draw.

UKNOWN/UNEXPECTED RISKS AND DISCOMFORTS

You may have a side effect that requires your researcher to take you off the study. You should contact your researcher immediately if you feel that you cannot tolerate participation in the study.

POSSIBLE BENEFITS OF THE STUDY

There is no guarantee that you will receive personal benefit from participating in this study, but it is possible that you may have some beneficial adaptations to training, such as increased strength and muscle mass. In addition, your participation may provide information that will increase the knowledge and information about low-intensity resistance training with BFR.

WITHDRAWAL FROM STUDY AND REFUSAL TO PARTICIPATE

Taking part in this clinical research study is voluntary and you can refuse to take part or stop at any time without stating a reason. Your withdrawal will not affect anything to which you would otherwise be entitled.

Special care will need to be taken when determining if you need to stop the study, with your health as the first priority. Your participation in this study may be stopped at any time by a) the researcher, b) the Institutional Review Board (a review group that gives approval to your study doctor to conduct this study), and (c) other appropriate regulatory agencies.

COST OF TREATMENT

All professional and diagnostic fees for tests and procedures that are part of this study will be provided free to you.

COMPENSATION FOR PARTICIPATION

There will be no compensation for participation in the study.

MEDICAL TREATMENT AND COMPENSATION FOR STUDY-RELATED INJURY

I understand that if I am injured in the course of this study, I alone may be responsible for the costs of treating my injuries. If I am injured (hurt) as a result of being in this study, the Kinesiology and Rehabilitative Science Department will give me immediate treatment needed for my injuries. I will then be told where I may get other treatment for

my injuries if needed. The cost of further treatment will be charged to my insurance company or to me. If my insurance company will not pay for these costs, they will be my responsibility. The Department has no program to compensate me in the form of money or anything else should I have an injury. You should immediately contact your researcher at the contact information shown below if you have any study-related illness or injury.

SOURCE FOR ADDITIONAL INFORMATION

If at any time between your visits you feel that any of your symptoms are causing you problems, or you have experienced a study-related injury, please contact your study doctor. The telephone number to reach your researcher or another authorized person is:

Dr. Ronald Hetzler or Morgan Kocher

1337 Lower Campus Rd PE/A 231, Honolulu, HI 96822

Phone: 808-956-7606

This voluntary consent form and study have been approved by an Independent Institutional Review Board (IRB) or Ethics Committee (EC). The IRB or EC is a group of scientific and non-scientific people who watch over research involving humans by following the guidelines and rules of the U.S. Food and Drug Administration. For any questions about your rights as a research subject, please direct inquiries to:

Human Studies Program

University of Hawaii

e-mail: uhirb@hawaii.edu

Phone: (808) 956-5007

GENERAL STATEMENT ABOUT PRIVACY

Records identifying you will be kept confidential and, to the extent permitted by applicable laws and/or regulations, will not be made publicly available. In the event of any publication regarding this study, your identity will remain confidential.

Representatives from government agencies, including institutional review boards, may need access to your original medical records and study records for the purpose of checking data collected for the study. By signing this consent form, you authorize this access.

Your coded study information and samples may also be used for additional unanticipated medical and/or scientific research projects in the future (but at all times in compliance with applicable law and regulation).

By signing this consent form you agree that you will not be able to have access to information about your participation in the study until the study is over. After that, you can obtain access to your information through your researcher.

AUTHORIZATION TO USE AND DISCLOSE RECORDS

For purposes of this study, the study doctor and the clinic will use medical information ("records") collected or created as part of the study that identifies you by name or in some other way, such as test results, identifiable blood or tissue samples, x-ray images, physical exam reports, medical history, and any other data collected or reviewed during the study. By signing this consent, you will permit the study doctor and the clinic to

obtain any of your records that they request for study purposes from your regular doctor and/or your other health care providers. You also have the choice of having some of your laboratory results that are available to the site sent to your private doctor. By signing, you agree that the study doctor and the clinic may use and share this information with the parties described below. You agree that, during the study, you may not see some of your records obtained or created as part of this study. You may be allowed to see this information once the study is finished.

Unless required by law, the study doctor and the clinic will share your records only with the study staff and other professionals involved in the study, and the Institutional Review Board (University of Hawaii Human Studies Program. Although efforts will be made to protect your privacy, absolute confidentiality of your records cannot be guaranteed, which may mean the information could be re-disclosed and not protected by federal privacy law.

You have the right to cancel your permission at any time by giving written notice to the researcher. If you cancel this authorization, then the researcher will no longer use or disclose your records, unless it is necessary to do so to preserve the scientific integrity of the study. Canceling this authorization will not affect previous or future uses of the information that that had been previously collected.

If you do not give your permission by signing this consent, or if you cancel this authorization later, you will not be able to participate in this study. Unless and until you do withdraw your permission, it will remain valid and effective.

AGREEMENT TO BE IN THE STUDY

By signing this informed consent form, I acknowledge that:

(1) I have carefully read and understand the information presented in this consent document.
(2) The purpose and procedures related to this research study have been fully explained to me and I have had the opportunity to ask questions and all of my questions were answered to my satisfaction.
(3) I have been informed of the parts of the program that are experimental and of the possible discomforts, symptoms, adverse events and risks that I might reasonably expect, and the possible complications, if any, that I might reasonably experience from both known and unknown causes as a result of my participation.
(4) I understand that I am free to withdraw this authorization and to discontinue my participation in this program any time. The consequences and risks, if any, of withdrawing from the program while it is ongoing have been explained to me.
(5) I understand that such withdrawal will not affect my ability to receive medical care to which I might otherwise be entitled.

Subject (or legally authorized representative)

_____

Subject Printed Name Signature Date

(or legally authorized representative)

_____

Description of Legal Representative's Authority (e.g., parent or legal guardian)

Person Obtaining Consent

_____

Printed Name & Title Signature Date

Appendix B: ACSM Absolute and Relative Contraindications to Resistance Training and Testing

Absolute

Unstable coronary heart disease

Decompensated heart failure

Severe pulmonary hypertension (mean pulmonary arterial pressure >55 mmHg)

Severe and symptomatic aortic stenosis

Acute myocarditis, endocarditis, or pericarditis

Uncontrolled hypertension (>180/110 mmHg)

Aortic dissection

Marfan syndrome

High intensity resistance training (80% to 100% of 1RM) in patients with active proliferative retinopathy or moderate or worse non-proliferative diabetic retinopathy

Relative

Major risk factors for coronary heart disease

Diabetes at any age

Uncontrolled hypertension (>160/100 mmHg)

Low functional capacity (<4 METs)

Musculoskeletal limitations

Individuals who have implanted pacemakers or defibrillators

Appendix C: Medical History Questionnaire

Name: _____ Date of Birth: _____ SID: _____

Date of Birth: _____ Age (years): _____ Ethnicity: _____

In Case of Emergency, whom may we contact?

Name: _____ Relationship: _____

Phone: _____

Do you participate in regular physical exercise? __ YES __ NO If yes, briefly describe:

_____

_____

_____

Have you previously participated in resistance training? __ YES __ NO

If yes, briefly describe:_____

_____

_____

Have you participated in regular resistance training in the past three months (at least 1 hr three times per week)? __ YES __ NO

Are you on any medication? ___ YES ___ NO If yes, briefly list the medication and the condition it is intended to treat:

_____

_____

_____

_____

_____

Appendix D: AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire

Assess your health status by marking all *true* statements

History

___a heart attack
___heart surgery
___cardiac catheterization
___coronary angioplasty (PTCA)
___pacemaker/implantable cardiac defibrillator/rhythm disturbance
___heart valve disease
___heart failure
___heart transplantation
___congenital heart disease

Symptoms

___You experience chest discomfort with exertion
___You experience unreasonable breathlessness
___You experience dizziness, fainting, or blackouts
___You experience ankle swelling
___You experience unpleasant awareness of a forceful or rapid heart rate
___You take heart medications

Other health issues

___You have diabetes
___You have asthma or other lung disease
___You have burning or cramping sensation in your lower legs when walking short distance
___You have musculoskeletal problems that limit your physical activity
___You have concerns about the safety of exercise
___You take prescription medication
___You are pregnant

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**

Cardiovascular risk factors

___You are a man ≥45 yr
___You are a woman ≥55 yr
___You smoke or quit smoking within the previous 6 months
___Your blood pressure is ≥140/90 mmHg
___You do not know your blood pressure
___You take blood pressure medication
___Your blood cholesterol level is ≥200 $mg \cdot dL^{-1}$
___You do not know your cholesterol level
___You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
___You are physically inactive (*i.e.*, you get <30 min of physical activity on at least 3 d per week)
___You have a body mass index ≥30 $kg \cdot m^{-2}$
___You have prediabetes
___You do not know if you have prediabetes

If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider as part of good medical care and progress gradually with your exercise program. You might benefit from using a facility with a **professionally qualified exercise staff** to guide your exercise program.

___None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guide program or

Appendix E: George Non-Exercise Test

Perceived Functional Ability (PFA)

Suppose you were going to exercise continuously on an indoor track for 1 mile. Which exercise pace is just right for you –not too easy and not too hard?

1 Walking at a slow pace (18 minutes per mile or more)
2 Walking at a slow pace (17-18 minutes per mile)
3 Walking at a medium pace (16-17 minutes per mile)
4 Walking at a medium pace (15-16 minutes per mile)
5 Walking at a fast pace (14-15 minutes per mile)
6 Walking at a fast pace (13-14 minutes per mile)
7 Jogging at a slow pace (12-13 minutes per mile)
8 Jogging at a slow pace (11-12 minutes per mile)
9 Jogging at a medium pace (10-11 minutes per mile)
10 Jogging at a medium pace (9-10 minutes per mile)
11 Jogging at a fast pace (8-9 minutes per mile)
12 Jogging at a fast pace (7-8 minutes per mile)
13 Running at a fast pace (7 minutes per mile or less)

How fast could you cover a distance of 3 miles and NOT become breathless or overly fatigued? Be realistic

1 I could walk the entire distance at a slow pace (18 minutes per mile or more)
2 I could walk the entire distance at a slow pace (17-18 minutes per mile)
3 I could walk the entire distance at a medium pace (16-17 minutes per mile)
4 I could walk the entire distance at a medium pace (15-16 minutes per mile)
5 I could walk the entire distance at a fast pace (14-15 minutes per mile)
6 I could walk the entire distance at a fast pace (13-14 minutes per mile)
7 I could jog the entire distance at a slow pace (12-13 minutes per mile)
8 I could jog the entire distance at a slow pace (11-12 minutes per mile)
9 I could jog the entire distance at a medium pace (10-11 minutes per mile)
10 I could jog the entire distance at a medium pace (9-10 minutes per mile)
11 I cold jog the entire distance at a fast pace (8-9 minutes per mile)
12 I could jog the entire distance at a fast pace (7-8 minutes per mile)
13 I could run the entire distance at a fast pace (7 minutes per mile or less)

Physical Activity Rating (PA-R)

Select the number that best describes your overall levels of physical activity for the previous 6 MONTHS:

0 avoid walking or exertion; e.g., always use elevator, drive when possible instead of walking

1 **Light activity**: walk for pleasure, routinely use stairs, occasionally exercise sufficiently to cause heavy breathing or perspiration

2 **Moderate activity**: 10 to 60 minutes per week of moderate activity; such as golf, horseback riding, calisthenics, table tennis, bowling, weight lifting, yard work, cleaning house, walking for exercise

3 **Moderate activity**: over 1 hour per week of moderate activity as described above

4 **Vigorous activity**: run less than 1 mile per week or spend less than 30 minutes per week in comparable activity such as running or jogging, lap swimming, cycling, rowing, aerobics, skipping rope, running in place, or engaging in vigorous aerobic-type activity such as soccer, basketball, tennis, racquetball, or handball.

5 **Vigorous activity**: run 1 mile to less than 5 miles per week, or spend 30 minutes to less than 60 minutes per week in comparable physical activity as described in 4 above.

6 **Vigorous activity**: run 5 miles to less than 10 miles per week or spend 1 hour to less than 3 hours per seek in comparable physical activity as described in 4 above

7 **Vigorous activity**: run 10 miles to less than 15 miles per week or spend 3 hours to less than 6 hours per week in comparable physical activity as described in 4 above

8 **Vigorous activity**: run 15 miles to less than 20 miles per week or spend 6 hours to less than 7 hours per week in comparable physical activity as described in 4 above

9 **Vigorous activity**: run 20-25 miles per week or spend 7 to 8 hours per week in comparable physical activity as described in 4 above

10 **Vigorous activity**: run over 25 miles per week or spend over 8 hours per week in comparable physical activity as described in 4 above

Appendix F: National Strength and Conditioning Association Estimated 1RM Table

Max Reps (RM)	1	2	3	4	5	6	7	8	9	10	12	15
%1RM	100	95	93	90	87	85	83	80	77	75	67	65
	10	10	9	9	9	9	8	8	8	8	7	7
	20	19	19	18	17	17	17	16	15	15	13	13
	30	29	28	27	26	26	25	24	23	23	20	20
	40	38	37	36	35	34	33	32	31	30	27	26
	50	48	47	45	44	43	42	40	39	38	34	33
	60	57	56	54	52	51	50	48	46	45	40	39
	70	67	65	63	61	60	58	56	54	53	47	46
	80	76	74	72	70	68	66	64	62	60	54	52
	90	86	84	81	78	77	75	72	69	68	60	59
	100	95	93	90	87	85	83	80	77	75	67	65
	110	105	102	99	96	94	91	88	85	83	74	72
	120	114	112	108	104	102	100	96	92	90	80	78
	130	124	121	117	113	111	108	104	100	98	87	85
	140	133	130	126	122	119	116	112	108	105	94	91
	150	143	140	135	131	128	125	120	116	113	101	98
	160	152	149	144	139	136	133	128	123	120	107	104
	170	162	158	153	148	145	141	136	131	128	114	111
	180	171	167	162	157	153	149	144	139	135	121	117
	190	181	177	171	165	162	158	152	146	143	127	124
	200	190	186	180	174	170	166	160	154	150	134	130
	210	200	195	189	183	179	174	168	162	158	141	137
	220	209	205	198	191	187	183	176	169	165	147	143
	230	219	214	207	200	196	191	184	177	173	154	150
	240	228	223	216	209	204	199	192	185	180	161	156
	250	238	233	225	218	213	208	200	193	188	168	163
	260	247	242	234	226	221	216	208	200	195	174	169
	270	257	251	243	235	230	224	216	208	203	181	176
	280	266	260	252	244	238	232	224	216	210	188	182
	290	276	270	261	252	247	241	232	223	218	194	189
	300	285	279	270	261	255	249	240	231	225	201	195
	310	295	288	279	270	264	257	248	239	233	208	202
	320	304	298	288	278	272	266	256	246	240	214	208
	330	314	307	297	287	281	274	264	254	248	221	215
	340	323	316	306	296	289	282	272	262	255	228	221
	350	333	326	315	305	298	291	280	270	263	235	228
	360	342	335	324	313	306	299	288	277	270	241	234
	370	352	344	333	322	315	307	296	285	278	248	241
	380	361	353	342	331	323	315	304	293	285	255	247

	390	371	363	351	339	332	324	312	300	293	261	254
Max Reps (RM)	1	2	3	4	5	6	7	8	9	10	12	15
%1RM	100	95	93	90	87	85	83	80	77	75	67	65
	400	380	372	360	348	340	332	320	308	300	268	260
	410	390	381	369	357	349	340	328	316	308	275	267
	420	399	391	378	365	357	349	336	323	315	281	273
	430	409	400	387	374	366	357	344	331	323	288	280
	440	418	409	396	383	374	365	352	339	330	295	286
	450	428	419	405	392	383	374	360	347	338	302	293
	460	437	428	414	400	391	382	368	354	345	308	299
	470	447	437	423	409	400	390	376	362	353	315	306
	480	456	446	432	418	408	398	384	370	360	322	312
	490	466	456	441	426	417	407	392	377	368	328	319
	500	475	465	450	435	425	415	400	385	375	335	325
	510	485	474	459	444	434	423	408	393	383	342	332
	520	494	484	468	452	442	432	416	400	390	348	338
	530	504	493	477	461	451	440	424	408	398	355	345
	540	513	502	486	470	459	448	432	416	405	362	351
	550	523	512	495	479	468	457	440	424	413	369	358
	560	532	521	504	487	476	465	448	431	420	375	364
	570	542	530	513	496	485	473	456	439	428	382	371
	580	551	539	522	505	493	481	464	447	435	389	377
	590	561	549	531	513	502	490	472	454	443	395	384
	600	570	558	540	522	510	498	480	462	450	402	390

Appendix G: Borg's Ratings of Perceived Exertion (RPE)

Rating	Perceived Exertion
6	No Exertion
7	Extremely Light
8	
9	Very Light
10	
11	Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal Exertion

Appendix H: Example SPSS Syntax

Three-Way Mixed Repeated Measures ANOVA

```
GLM Pre_Pre_Plasma_TSA Pre_Post1_Plasma_TSA Pre_Post2_Plasma_TSA Pre_24Post_Plasma_TSA
    Post_Pre_Plasma_TSA Post_Post1_Plasma_TSA Post_Post2_Plasma_TSA Post_24Post_Plasma_TSA BY
    Training_Status
  /WSFACTOR=Session 2 Polynomial Time 4 Polynomial
  /METHOD=SSTYPE(3)
  /PLOT=PROFILE(Time*Session*Training_Status)
  /EMMEANS=TABLES(Training_Status) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Session) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Time) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Training_Status*Session)
  /EMMEANS=TABLES(Training_Status*Time)
  /EMMEANS=TABLES(Session*Time)
  /EMMEANS=TABLES(Training_Status*Session*Time)
  /PRINT=DESCRIPTIVE ETASQ OPOWER HOMOGENEITY
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Session Time Session*Time
  /DESIGN=Training_Status.
```

Two by Two Repeated Measures ANOVA

```
GLM EPOC_KCAL_PRE EPOC_KCAL_Post BY Training_Status
  /WSFACTOR=Session 2 Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Training_Status) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Session) COMPARE ADJ(LSD)
  /EMMEANS=TABLES(Training_Status*Session)
  /PRINT=DESCRIPTIVE ETASQ OPOWER HOMOGENEITY
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Session
  /DESIGN=Training_Status.
```